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## LYMPHOID TUMORS IN MICE RECEIVING ESTROGENS

W U GARDNER, PH D

A KIRSCHBAUM, PH D

AND

L C STRONG, PH D, Sc D

NEW HAVEN, CONN

The occurrence of mammary and uterine tumors in mice receiving estrogens has been attributed, at least in part, to the specific hyperplasia of the genital tissues occurring under such stimulation. The origin of spindle cell sarcoma at or near the site of injection indicates that estrogen might act on nongenital mesenchymal tissues much as the synthetic carcinogens do<sup>1</sup>. The incidence of such tumors is low, however, and the significance of the observations may be questioned<sup>2</sup>.

Lymphoid tumors frequently occur spontaneously in certain strains of mice. In other strains the incidence is low. Such tumors developed in 3 of 111 mice of three different strains following injections of estrogen, while such tumors did not appear among the controls<sup>3</sup>. One of these tumors was definitely leukemic<sup>4</sup>. Two others arose as mediastinal masses and invaded the surrounding tissues. Similar tumors were observed in 14 mice from several different strains given estrogens<sup>5</sup>. Such tumors did not appear in untreated mice of these strains.

From the Department of Anatomy, Yale University

This investigation has been supported by the Jane Coffin Childs Memorial Fund for Medical Research and the Anna Fuller Fund

1 (a) Gardner, W U, Smith, G M, Strong, L C, and Allen, E. *Arch Path* **21** 504, 1936. (b) Lacassagne, A. *Compt rend Soc de biol* **126** 190, 1937. (c) Burns, E L, Sontzeff, V, and Loeb, L. *Am J Cancer* **32** 534, 1938.

2 Gardner, W U. *Arch Path* **27** 138, 1939. Burns, Sontzeff and Loeb<sup>1c</sup>.

3 Gardner, W U, in *Some Fundamental Aspects of the Cancer Problem* Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Science, New York, Science Press, 1937, p 67, Science, 1937, supp 4, p 67.

4 Lawrence, J H, and Gardner, W U. *Am J Cancer* **33** 112, 1938.

5 Lacassagne, A. *Compt rend Soc de biol* **126** 193, 1938.

## MATERIALS AND METHODS

Mice of the C<sub>3</sub>H strain were used in the present investigation. This strain had been inbred by brother to sister matings for at least thirty-six generations at the time young mice were removed for this study.<sup>6</sup> The control mice were from unselected representatives of the same generations. The treated animals include those of all groups from which the majority have been submitted to autopsy at this time and which have not been previously reported on.

The mice were maintained on a diet of Purina fox chow. At the start of each experiment 6 mice were usually placed in each cage. Food and water were available at all times.

Estradiol dipropionate, estradiol benzoate, equilin benzoate, cholesterol, cholesterol benzoate, carotene, testosterone propionate, testosterone propionate and estradiol benzoate<sup>7</sup> given simultaneously, and sesame oil given alone were administered to variable numbers of mice (tables 1 and 2). The chemicals administered, with the exception of colchicine, were dissolved in sesame oil. All injections were made subcutaneously on the dorsal part of the body. The amount of material given at each injection was usually 0.05 cc. The injections were started in mice 3 to 140 days of age and continued at biweekly, weekly, fortnightly or longer intervals throughout life or in some cases for limited periods of six to ten weeks.

## OBSERVATIONS

Three hundred and fifty-two mice of the C<sub>3</sub>H strain have been observed in the experiments reported here. Of these, 149 received estrogens in variable amounts throughout their lives, starting at ages ranging from 3 to 140 days. The observations on these mice are grouped according to treatment in table 1.

Estradiol dipropionate was administered to 12 mice (6 males and 6 females), starting at 34 to 36 days of age. These animals were given weekly injections of 50 micrograms subcutaneously. All the animals lived to be 150 days of age or older. One is living at 300 days of age. Of the 11 mice which died, 7 had lymphoid tumors at ages ranging from 175 to 272 days, after a total injection of 12 to 17 mg. of estradiol dipropionate.

In all of these mice the mediastinum was extensively infiltrated with lymphoid tissue. All of them presented respiratory difficulties prior to death. A white irregular mass of tissue filled the mediastinum from the manubrium to the diaphragm, invading the intercostal muscles, the pericardium, heart muscle, lungs and in some cases the esophagus, large arteries and trachea. The tumors were confined entirely to the

---

6 Strong, L. C. *Genetics* **20** 586 1935

7 The estradiol dipropionate was supplied by Dr. E. Oppenheimer, of Ciba Pharmaceutical Products, Inc. The estradiol benzoate (progynon B) and testosterone propionate were supplied by Drs. E. Schwenk and M. Gilbert, of the Schering Corporation. The equilin benzoate was supplied by Dr. A. Girard, of Paris, the cholesterol benzoate was prepared by Dr. W. Bergmann, the carotene was obtained from the British Drug Houses Ltd., London, and the cortical extract was supplied by Dr. G. F. Cartland, of the Upjohn Company.

mediastinum in 4 mice. In 1 mouse a white blood cell count made two days prior to death revealed a subnormal level of 2,400 cells per cubic millimeter. In 3 other mice the spleen and lymph nodes were also involved, and the ovaries in 2 females. Sections of the liver and genital

TABLE 1—Incidence of Lymphoid Tumors in C<sub>3</sub>H Mice Receiving Estrogens

Treat- ment*	Mice	Number Living	Number Dying Over 150 Days of Age	Number with Lymphoid Tumors	Age at Appearance of Lymphoid Tumors	Age Range of Mice Without Tumors	Amount of Material,† Micrograms
DPB	12	1	11	7	$\begin{Bmatrix} 175 & 247 \\ 207 & 270 \\ 209 & 272 \\ 236 \end{Bmatrix}$	209-294	50
ABG	6	0	4	1	310	284-366	16.6
EBC	6	0	6	0		189-445	100
EB	16	0	16	4	$\begin{Bmatrix} 478 \\ 316 \\ 387 \\ 306 \end{Bmatrix}$	191-478	100
B	16	0	13	1	345	167-504	33.3
B	25	5	19	1	309	145-645	$\frac{33.3}{4}$
B	12	0	12	1	517	205-576	$\frac{16.6}{4}$
B	6	0	6	0		319-572	$\frac{16.6}{3}$
B	10	0	10	0		237-479	$\frac{16.6}{2}$
B	13	0	13	1	360	220-528	8.3
B	17	0	17	2	$\begin{Bmatrix} 272 \\ 290 \end{Bmatrix}$	239-392	16.6
Colchicine B	10	0	9	4	$\begin{Bmatrix} 487 \\ 444 \\ 412 \\ 346 \end{Bmatrix}$	153-487	$\begin{Bmatrix} 25 \text{ colchicine} \\ \text{times 11} \\ 16.6 \end{Bmatrix}$
Totals	149	6	136	22			

\* DPB = estradiol dipropionate, ABG = carotene and estradiol benzoate, EBC = equilin benzoate and extract of adrenal cortex, EB = equilin benzoate, B = estradiol benzoate.

† The amount stated was given to each mouse weekly unless written as  $\frac{33.3}{4}$ , which means 33.3 micrograms every fourth week.

tissues of the aforementioned 3 mice revealed general lymphoid invasion of all organs. Blood cell counts were not made immediately before autopsy in these cases. The bone marrow was almost entirely replaced by compact bone as occurs in mice receiving estrogens, so that the extent of myeloid invasion could not be determined.

Six mice were given weekly injections of 0.5 mg of carotene and 16.6 micrograms of estradiol benzoate. Four of these mice lived for

150 days or more. One at autopsy showed enlargement of all lymph nodes. The liver, spleen and other tissues studied showed lymphoid infiltration.

Six mice were given weekly injections of 100 micrograms of equilin benzoate and 0.05 cc of an active extract of adrenal cortex daily for one hundred and eighty-nine to four hundred and forty-five days. In none of these mice did lymphoid tumors develop. Lymphoid tumors developed in 4 of 16 mice given weekly injections of 0.1 mg of equilin benzoate weekly for periods of one hundred and ninety-one to four hundred and seventy-eight days. Blood counts were not made on any of these mice, but all showed extensive lymphoid involvement of the mediastinum, lymph nodes and spleen. Lymphoid cells had also infiltrated other tissues, particularly the liver and kidneys. Splenic and mediastinal tissues from 2 of these mice were grafted into other mice of the same strains, the lymphoid tumors which developed were carried for many generations.<sup>8</sup>

Of 16 mice given 33.3 micrograms of estradiol benzoate weekly, 13 lived to be 150 days old or older. In 1 male mouse lymphoid leukemia developed, and the mouse was killed at 345 days of age. Only 2 mice in the group lived to an age of 345 days (347 and 504). In a mouse which received an injection of 33.3 micrograms of estradiol benzoate every fourth week a mammary tumor developed at 266 days of age, and the mouse when killed at 309 days of age showed marked enlargement of the spleen and lymph nodes. Study of dry imprints of these tissues stained with May-Grunwald-Giemsa stain and of the sectioned tissues proved the presence of a lymphoid tumor. Eighteen other mice, 17 of which lived to 150 days or more, died without showing lymphoid tumors. In another mouse given 16.6 micrograms of estradiol benzoate every fourth week a lymphoid tumor developed at 517 days of age, while in 11 other animals living to from 205 to 576 days of age no such tumors developed.

In none of 16 mice which received 16.6 micrograms of estradiol benzoate every second or third week did lymphoid tumors develop though they lived to from 237 to 572 days of age. Ten of the 16 mice died with mammary tumors.

One of 13 mice given 8.3 micrograms of estradiol benzoate weekly died with a lymphoid tumor at 360 days of age. A mammary tumor had developed in this mouse at 267 days of age. It was removed and recurred but did not reappear following a second operation. At autopsy a large invasive mediastinal tumor was found. The spleen and the mesenteric and other abdominal nodes were all greatly enlarged.

---

<sup>8</sup> Lits, F., Kirschbaum, A., and Strong, L. C. *Am J Cancer* **34** 196, 1938

In 6 of 27 mice treated with 166 micrograms of estradiol benzoate weekly lymphoid tumors developed. Four of 10 mice which showed tumors received eleven weekly injections of 25 micrograms of colchicine in aqueous solution in addition to the estrogen. These tumors showed the same general range of structure as those in the preceding groups. One was transplanted and maintained for two generations.

*Incidence of Leukemia in Control Mice of the C<sub>3</sub>H Strain*—Included in the group listed as control mice were untreated male and

TABLE 2—*Incidence of Lymphoid Tumors in Untreated Mice of the C<sub>3</sub>H Strain and in Mice Not Treated with Estrogens, Treated with Lower Amounts of Estrogen or Given Estrogen and Androgen Simultaneously*

Treat ment*	Mice	Number Living	Number Dying Over 150 Days of Age	Number with Lymphoid Tumors	Age at Appearance of Lymphoid Tumors	Age Range of Nonleukemic Mice	Amount of Material, Milligrams (TP) or Micrograms (B)
TP	11	0	11	0		257-479	1.25 2.5
TPO	17	0	17	1	492	181-533	16.6-33.3 B 1.25-2.5 TP
OTP	30	12	18	0		173-461	0.5-1.0 TP 8.3-33.3 B
A	6	0	6	0		233-520	
B	12	0	12	0		227-284	33.3† 2 × 6
B	6	0	6	0		274-509	33.3 3 × 3
B	9	0	9	0		165-299	3.3
B	12	0	12	0		223-567	0.033
C	10	0	10	0		297-618	0.5 mg
CB	12	0	12	0		152-646	0.5 mg
C <sub>3</sub> H breeders	40	0	40	0		208-577	
OB	38	0	38	0		247-614	0.05 cc
Total	203	12	191	1			

\* TP = testosterone propionate, TPO and OTP = testosterone propionate plus estrogen. A = carotene, B = estradiol benzoate, C = cholesterol, CB = cholesterol benzoate, OB = sesame oil.

† — means 33.3 micrograms of estradiol benzoate every second week for six injections, 2 × 6 after which treatment was stopped.

female mice which had been used as breeders, mice which had received weekly injections of sesame oil, cholesterol, cholesterol benzoate or carotene, injections of estrogens in low amounts or briefly, or injections of testosterone or of testosterone and estrogens simultaneously. These groups consist of all of those in which one half or more of the mice have been examined post mortem at the time of writing (table 2).

Lymphoid tumors were not observed in any of the 117 mice which had received injections of the nonestrogenic substances or had been left untreated. In none of the 39 mice which had received weekly injec-



tions of 3.33 micrograms of estradiol benzoate or less or short term injections of larger amounts of estrogens did lymphoid tumors develop. A lymphoid tumor developed in 1 of 47 mice which received simultaneous injections of estradiol benzoate and testosterone propionate. This mouse had received weekly injections of 16.6 micrograms of estradiol benzoate and 2.5 mg of testosterone propionate for four hundred and thirty-one days. Injections were started at the age of 61 days. The mouse died with a large cervical or vaginal tumor. All subcutaneous and abdominal nodes were enlarged and white. Histologic study and an examination of dry imprints of the nodes revealed a well developed lymphoid neoplasm.

#### COMMENT

Lymphoid tumors may occur in mice, with leukemic involvement of the blood stream or as localized overgrowths of lymphoid tissue with or without diffuse metastases or general involvement of all lymphoid tissues. Three factors have been observed to increase the incidence of such tumors or to be associated with such an increase, namely, a genetic factor,<sup>9</sup> roentgen irradiation<sup>10</sup> and the carcinogenic chemical methylcholanthrene.<sup>11</sup> Other factors have likewise been claimed by various investigators to increase the incidence of lymphoid tumors.<sup>12</sup>

Among many strains of mice lymphoid tumors appear to a limited extent. The incidence in the C<sub>3</sub>H strain used in this investigation appears to be very low, however. Considering the 117 untreated or nonestrogen-treated mice reported on, the incidence is quite insignificant. Lymphoid tumors have occurred in mice of this strain, but the incidence appears to be less than 1 per cent.<sup>13</sup> The presence of 22 such tumors among 149 mice receiving estrogens, or in 15.4 per cent, suggests that the treatment received by these animals had some stimulating effect on the appearance of lymphomatosis. The incidence of lymphomatosis and myelosis in mice subjected to roentgen irradiation was 23.5 per cent, but that of the untreated control mice studied by Furth and Furth<sup>10</sup> was 6.2 per cent. Though based on smaller groups of estrogen-treated mice, the increased incidence of lymphoid neoplasms in the mice reported here (0-15.4 per cent) appears of comparable significance. Myelosis, however, was not observed in mice of the C<sub>3</sub>H strain.

The estrogens have been associated with the development of tumors of the genital tissues, it is assumed, by specific growth-stimulating effects on such tissues. The mesenchymal hemopoietic tissues have not been

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9 Richter, M. N., and MacDowell, E. C. *Physiol Rev* **15** 509, 1935.

10 Furth, J., and Furth, O. B. *Am J Cancer* **28** 54, 1936.

11 Morton, J. J., and Mider, G. B. *Science* **87** 327, 1938.

12 Richter, M. N. *Leucemia*, in Downey, H. *Handbook of Hematology* New York, Paul B. Hoeber, Inc., 1938, chap. 42. Richter and MacDowell.<sup>9</sup>

13 Strong, L. C. Unpublished data.

generally assumed to be markedly affected by the "sex hormones" The incidence of lymphatic leukemia, though transmitted to a greater extent by the female than the male, shows no tendency toward limitation to that sex as does the incidence of mammary tumor<sup>9</sup>

Certain recent experiments, however, demonstrated that estrogens affect the myeloid tissue of the bone marrow in dogs<sup>14</sup> and chickens<sup>15</sup> In the former species agranulocytosis developed and finally anemia, which invariably terminated in death Similar changes occurred in certain mice receiving estrogens in large amounts<sup>16</sup> Also in mice as in birds, the bone marrow became largely replaced by osseous tissue<sup>17a b c</sup> Whether any of these changes can be associated with the development of lymphoid tumors is, however, problematic Myelogenous leukemias were not observed In mice bony changes were prevented by simultaneous administration of adequate amounts of testosterone propionate<sup>17d</sup> One mouse so treated in this series presented a lymphoid tumor

Malignant lymphomatosis occurred in all strains of mice which tolerated estrogens in large amounts in our laboratory The number of mice tested and the number of control mice on which observations were completed in several other strains are inadequate at this time to warrant definite conclusions other than that the tendency described in this paper apparently is not limited to strain

#### SUMMARY

Twenty-two mice (15.4 per cent) of the C.H strain showed lymphoid tumors, leukemic or nonleukemic, among a group of 149 which had received estrogens in variable amounts starting at ages of 3 to 140 days and continuing until death

None of 117 untreated or nonestrogen-treated mice of this same strain showed lymphoid tumors A lymphoid tumor developed in 1 of 86 mice receiving brief estrogenic treatment or estrogen and testosterone propionate simultaneously

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14 (a) Tislowitz, R. *Acta brev Neerland* **13** 183, 1938 (b) Gardner, W. U., and others. Unpublished data

15 Landauer and others. Unpublished data

16 Unpublished data

17 (a) Gardner, W. U., and Pfeiffer, C. A. *Proc Soc Exper Biol & Med* **37** 678, 1938, (b) **38** 599, 1938 (c) Pfeiffer, C. A., and Gardner, W. U. *Endocrinology* **23** 485, 1938 (d) Landauer, W., Pfeiffer, C. A., Gardner, W. U., and Mann, E. B. *Proc Soc Exper Biol & Med* **41** 80, 1939

# PRODUCTION OF INTERNAL TUMORS WITH CHEMICAL CARCINOGENS

H P RUSCH, M D  
AND  
C A BAUMANN, PH D  
MADISON, WIS  
AND  
G L MAISON, M D  
ST LOUIS

The carcinogenic activity of various chemicals has usually been studied on skin or on subcutaneous tissue, but neoplasms have also been induced in internal organs with these agents. The internal tumors produced may be considered in three groups, according to the methods employed. The first group includes those produced by the use of carcinogenic agents which have a specific affinity for the tissues involved. Thus aminoazotoluene when fed or injected subcutaneously produces tumors in the liver rather than at the site of application. The evidence is summarized by Shear <sup>1</sup> and by Cook and Kennaway <sup>2</sup>. Betanaphthylamine, like aminoazotoluene, produces tumors of a specific tissue, although it may be in contact with other cells at a greater concentration. Its administration, either oral or subcutaneous, results in the formation of tumors of the bladder <sup>3</sup>. Certain derivatives of aminoazotoluene likewise produce tumors of the bladder <sup>4</sup>. Although the chief site of action of aminoazotoluene is the liver, the dye also affects the thyroid, producing degenerative atrophy followed by epithelial metaplasia, which closely resembles squamous cell epithelioma <sup>5</sup>.

The second group of internal tumors are those induced by carcinogenic chemicals which are most effective at the site of application but which also produce neoplasms at distant sites that may be more or less susceptible to spontaneous development of tumor. Thus, when dibenzanthracene was injected subcutaneously into strain C mice, the

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From the Department of Physiology, University of Wisconsin Medical School

1 Shear, M J. Am J Cancer **29** 269, 1937

2 Cook, J W, and Kennaway, E L. Am J Cancer **33** 50, 1938

3 Hueper, W C. Arch Path **25** 856, 1938

4 Yoshida, T. Gann **29** 295, 1935. Otsuka, I, and Nagao, N. *ibid* **30** 561, 1936. Nagao, N. *ibid* **31** 335, 1937. Zylberszac, S. Compt rend Soc de biol **125** 389, 1937

5 Yoshida, T. Tr Jap Path Soc **22** 934, 1932

incidence of pulmonary tumors rose from "moderate" to "high", in strain A mice it rose from "high" to "very high" <sup>6</sup> Tumors of the liver, also, have occasionally been produced by the subcutaneous injection of chemicals whose chief center of activity is the area of application Andervont and Lorenz <sup>7</sup> observed hematomas in 8 of 17 survivors when dibenzanthracene was injected subcutaneously into 40 C<sub>3</sub>H mice Strong, Smith and Gardner <sup>8</sup> induced 8 hepatomas in 42 CBA mice by injecting 3,4,5,6-dibenzcarbazole Boyland and Brues <sup>9</sup> had previously reported hypertrophic changes in the bile ducts of 80 per cent of mice receiving this agent applied to the skin Furth and Furth <sup>10</sup> observed hemangioma of the liver in 2 of 96 mice when benzpyrene was injected into the spleen

The third group of internal tumors are those produced by direct contact between the carcinogenic agent and the tissue from which the tumor arises This method has yielded tumors in most of the tissues and with most of the carcinogenic agents tried The available information is listed in table 1 <sup>11</sup> A variation of this method is of special interest The feeding of carcinogenic hydrocarbons has resulted in occasional tumors of the upper part of the digestive tract (table 1), but tumors of the lower part of the tract have not been reported Furthermore, some investigators have failed to observe any tumors when dibenzanthracene or benzpyrene was fed for periods of six to ten months

6 Andervont, H B, cited by Fieser, L Am J Cancer **34** 37, 1938

7 Andervont, H B, and Lorenz, E Pub Health Rep **52** 637, 1937

8 Strong, L C, Smith, G M, and Gardner, W U Yale J Biol & Med **10** 335, 1938

9 Boyland, E, and Brues, A M Proc Roy Soc, London, s B **122** 429, 1937

10 Furth, J, and Furth, O B Am J Cancer **34** 169, 1938

11 (a) Brunswick, A, and Bissell, A D Arch Surg **36** 53, 1938 (b) Brunswick, A Am J Cancer **34** 540, 1938 (c) Oberling, C, Guerin, M, and Guerin, P Compt rend Soc de biol **123** 1152, 1936 (d) Weil, A Arch Path **26** 777, 1938 (e) Valade, P Compt rend Acad d sc **204** 1281, 1937 (f) Bull Assoc franç p l'étude du cancer **26** 452, 1937 (g) Athias, M Compt rend Soc de biol **126** 585, 1937 (h) Campbell, J A Brit J Exper Path **15** 287, 1934 (i) Seelig, M G, and Benignus, E L Am J Cancer **28** 96, 1936 (j) Nakahara, W, and Fujiwara, T Gann **31** 568 and (k) 660, 1937 (l) Berenblum, I, and Kendal, L P Biochem J **30** 429, 1936 (m) Boyland, E, and Burrows, H J Path & Bact **41** 231, 1935 (n) Burrows, H Proc Roy Soc, London, s B **111** 238, 1932 (o) Roussy, G, Oberling, C, and Guerin, M Bull Acad de med, Paris **112** 809, 1934 (p) Van Prohaska, J, Brunswick, A, and Wilson H Arch Surg **38** 328, 1939 (q) Waterman, N Acta brev Neerland **7** 18, 1937 (r) Bonne, C Ztschr f Krebsforsch **25** 1, 1927 (s) Voronoff, S, and Alexandrescu, G Neoplasmes **8** 129, 1929 (t) Bagg, H J Am J Cancer **26** 69, 1936

On the other hand, the feeding of certain samples of wheat germ oil to rats has resulted in the formation of tumors of the peritoneum in from thirteen to two hundred days<sup>12</sup> The oils were obtained by extrac-

TABLE 1—*Tumors Induced by Duct Injection*

Tissue	Agent	Animals	Number with Tumor	Type of Tumor	Reference
Bone	Benzpyrene	12 mice	1	Osteosarcoma	11a
	Methylcholanthrene	33 rats	4	Fibrosarcoma	11b
Brain	Benzpyrene	11 rats	3	Hypophysial adenoma	11c
			1	Epithelial tumor of anterior lobe of pituitary	11c
	1,2,5,6 dibenzanthracene	1 rat	1	Carcinoma	11d
	Styryl 430	3 rats	3	Glioma, meningioma and glioma of ependyma	11d
Esophagus	Methylcholanthrene	50 rats	5	Rhabdomyosarcoma	11e f
Heart	Methylcholanthrene	33 guinea pigs	1	Polymorphous cell sarcoma of auricle	11g
Kidney	Dibenzanthracene	7 rats	2	Epidermoid carcinoma	20
		51 mice	10		
Liver	Dibenzanthracene	56 mice	2	Carcinoma?	20
Lung	Road tar dust	100 mice	61	Adenoma and adenocarcinoma	11h
	Coal smoke soot	100 mice	8	Carcinoma	11i
Peritoneum	Benzpyrene	27 mice	19	Polymorphous and spindle cell sarcoma	11j
	Methylcholanthrene	40 mice	9	Spindle cell sarcoma	11k
	Dibenzanthracene	80 mice	5	Spindle cell sarcoma	11l
	Dibenzanthracene	20 mice	5	Sarcoma	11m
		38 rats	1		
	Dibenzanthracene	10 rats	8	Spindle cell sarcoma	11n
	Thorium dioxide	10 rats	4	Fibrosarcoma	11o
Prostate	Benzpyrene	50 rats	37	Squamous cell epithelioma	19
			4	Sarcoma	19
Spleen	Methylcholanthrene	1 mouse	1	Fibrosarcoma	20
	Benzpyrene	96 mice	2	Sarcoma	10
	Benzpyrene	96 mice	2	Hemangioma	10
Stomach	Methylcholanthrene (fed)	48 mice	2	Papilloma	11p
	Benzpyrene (fed)	6 mice	5	Squamous cell carcinoma	11q
	Tar (painted on mouth)	50 rats	17	Papilloma of fore stomach	11r
	Tar wool fat, aniline and toluenediamine	10 rats	1	Carcinoma	11s
Testis	Zinc chloride	1 chicken	Small per cent	Teratoma	11t
Trachea	Methylcholanthrene	50 rats	8	Sarcoma	11e f
Uterus	Dibenzanthracene	21 mice	1	Epidermoid carcinoma	20

12 Rowntree L G Steinberg, A , Dorrance, G, and Ciccone, E F Am J Cancer 31 359, 1937

tion of the wheat germ with ether. Purified wheat germ oils were devoid of carcinogenic activity. Carruthers<sup>13</sup> and Halter<sup>14</sup> were unable to produce tumors by feeding wheat germ oil.

✧ The present report deals with the action of carcinogenic agents on the spleen, liver, submaxillary glands, testis, epididymis, uterus and bone marrow and the submucosa of the stomach and duodenum. Furthermore, an attempt was made to confirm the production of tumors of the peritoneum by the feeding of wheat germ oil.

#### PROCEDURE

Both mice and rats were used in these experiments. The mice were males of strains A, C and C<sub>3</sub>H, and were 8 to 10 weeks of age. The rats were 13 to 15 weeks of age. The organs to be studied were exposed surgically and either 3,4-benzpyrene or 1,2,5,6-dibenzanthracene was injected. Solutions of the carcinogenic agents in corn oil were used: 10 mg per cubic centimeter of benzpyrene or 5 mg per cubic centimeter of dibenzanthracene. The injections were made deeply with a narrow gage needle to minimize leakage on withdrawal. Usually only one injection was made, but in a few instances a second was given. The exact doses injected into the various organs are listed in table 2.

Injections into the bone marrow were made at weekly intervals for eighteen weeks. For this purpose a hole slightly smaller than a no. 25 needle was drilled through the upper third of the tibia. From 0.05 to 0.10 cc of the carcinogenic solution was injected at one time. Immediately after withdrawal of the needle the opening was plugged with bone wax to prevent leakage. One tibia received an injection one week, the other the next, thus allowing two weeks between injections into any one bone. Control rats received similar injections of corn oil. Complete blood counts were made before the introduction of the carcinogenic agent and at weekly intervals for four months; thereafter examinations of the blood were made at monthly intervals.

The effect of wheat germ oil in the diet on the production of tumors was studied as follows. Fifty young adult male mice of strain A were divided into two groups of 25 each. One group received a diet consisting of 93 parts of Steenbock stock ration plus 7 parts of wheat germ oil; the other group received the same diet with 0.05 per cent aminoazotoluene added. In addition, 6 male rats 2 to 3 months of age received a diet containing 75 parts of Steenbock stock diet and 25 parts of wheat germ oil. The wheat germ oil was prepared by extracting wheat germ with ethyl ether in a continuous extractor and evaporating the ether under reduced pressure. The residue was fed without further purification.

#### RESULTS OF THE INJECTION OF HYDROCARBONS INTO VARIOUS TISSUES

*Bone Marrow*—Although as much as 13 mg of benzpyrene and 7.25 mg of dibenzanthracene were injected into the bone marrow, the animals remained in good physical condition. The marrow in the long bones, however, became markedly hyperplastic. In 3 of 4 rats living

13 Carruthers, C. Proc Soc Exper Biol & Med **40** 107, 1939.

14 Halter, C. R. Proc Soc Exper Biol & Med **40** 257, 1939.

over forty weeks had firm tumors developed at the site of injection. Two had received benzpyrene, the other, dibenzanthracene. The histologic appearance of the tumors differed somewhat, but all were classified

TABLE 2—*Injection of Carcinogenic Agents into Various Tissues*

Animals Used	Strain	Organ	Agent Injected	Total Amount, Mg	Tumor Noted	Length of Life After Injection, Mo	Number and Type of Tumors
9 rats	Stock	Liver	1,2,5,6 dibenzanthracene	1.25-2.62	None	9-17	
9 rats	Stock	Spleen	1,2,5,6 dibenzanthracene	1.4	None*	9-17	
13 rats	Stock	Testis	1,2,5,6 dibenzanthracene	1.3	None	9-17	
6 rats	Stock	Epididymis	1,2,5,6 dibenzanthracene	1	None	9-15	
3 rats	Stock	Submaxillary gland	1,2,5,6 dibenzanthracene	1.15	1 at 15 mo 1 to 16 mo	12-16	2 spindle cell sarcomas
5 rats	Stock	Bone marrow	1,2,5,6-dibenzanthracene	1.75-7.25	1 at 12 mo	4-13	1 fibrosarcoma
5 rats	Stock	Bone marrow	3,4 benzpyrene	0.5-1.3	2 at 9 mo	4-11	2 fibrosarcomas
5 rats	Stock	Submucosa of stomach	3,4 benzpyrene	1.5-2	1 at 7 mo 1 at 13 mo 1 at 15 mo	7-16	1 spindle cell sarcoma 1 myoma 1 adenocarcinoma
2 rats	Stock	Submucosa of duodenum	3,4 benzpyrene	1.5-2	None	13-17	
4 rats	Stock	Uterine horn	3,4 benzpyrene	1.2	1 at 12 mo	9-16	1 myogenic sarcoma
2 rats	Stock	Spleen	3,4 benzpyrene	1.5	None	7 & 17	
1 rat	Stock	Submaxillary gland	3,4 benzpyrene	1	None	14	
3 mice	C <sub>3</sub> H	Liver	1,2,5,6 dibenzanthracene	0.25	None	6-14	
2 mice	C <sub>3</sub> H	Spleen	1,2,5,6 dibenzanthracene	0.25	1 at 6 mo	6	1 spindle cell sarcoma
3 mice	C <sub>3</sub> H	Testis	3,4 benzpyrene	0.2	None	6	
2 mice	C <sub>3</sub> H	Submaxillary gland	3,4 benzpyrene	0.5	1 at 5½ mo	6½	1 spindle cell sarcoma
9 mice	"A"	Spleen	3,4 benzpyrene	0.3-1.3	None	7-14	
8 mice	"A"	Testis	3,4 benzpyrene	0.3-0.5	None	4-9	
15 mice	"A"	Submaxillary gland	3,4 benzpyrene	0.3-0.7	10 at 2-3½ mo	4-7	10 squamous cell carcinomas 2 squamous cell carcinomas
4 mice	"C"	Submaxillary gland	3,4 benzpyrene	0.3-0.5	2 at 3 mo	3-6	

\* One spindle cell sarcoma of subcutaneous tissue of the back occurred at sixteen months.

as periosteal fibrosarcoma (fig. 1). The tumors were invasive and grew to a large size (one weighed 75 Gm. at autopsy), but no metastases were found.

The blood picture was unchanged except for occasional slight increases in the number of lymphocytes, due possibly to mild infections. The blood count remained remarkably constant. This result differs

sharply from that of workers who have observed changes in the blood stream due to the administration of carcinogenic agents Barnes and Furth<sup>15</sup> and Furth and Furth<sup>10</sup> observed leukemia following injections of benzpyrene into the spleens of mice Lanza<sup>16</sup> observed leukemia in rats after benzpyrene was injected into the bone marrow Erythro-leukemia has been reported in 50 per cent of a series of rats receiving

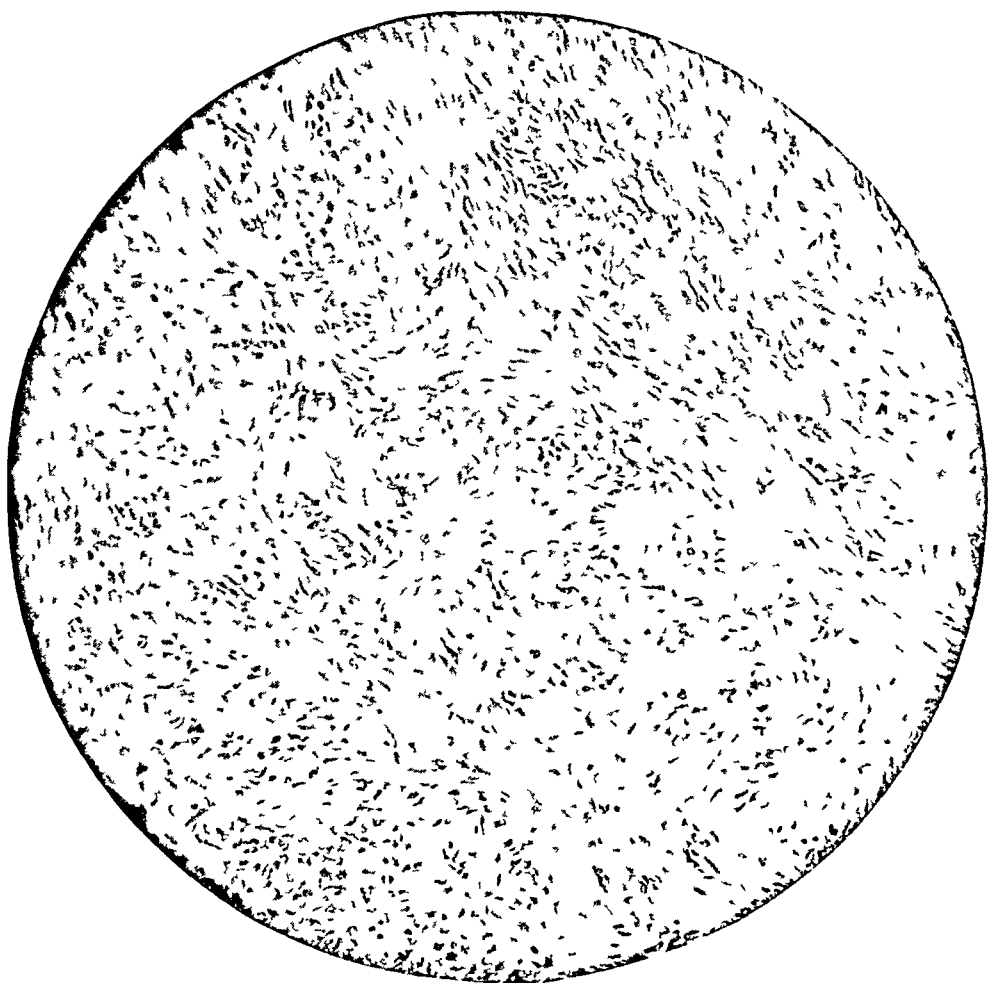


Fig 1—Periosteal fibrosarcoma induced by injections of benzpyrene into the bone marrow of a rat

injections of tar in the bone marrow<sup>17</sup> Changes occurred in twenty to sixty days, and, once initiated, proceeded in the absence of further injections Changes in the blood resembling leukemia have also been

15 Barnes, W A, and Furth, J Am J Cancer 30 75, 1937

16 Lanza, G Pathologica 30 185, 1938

17 Bernard, J Sang 8 28, 1934



induced in mice by subcutaneous injections of sodium 1,2,5,6-dibenzanthracene-9-10-endoalphetasuccinate<sup>18</sup>

There is thus abundant evidence that carcinogenic agents do produce changes in the blood stream. Possibly our animals were unusually resistant to leukemic changes, since they actually received larger amounts of carcinogenic agents than were employed by other investigators. Moreover, since some strains of animals exhibit a much higher incidence of spontaneous leukemia than others, it is quite likely that the tendency toward induced leukemia also varies with the strain.

*Submaxillary Gland*—Squamous cell carcinoma of the submaxillary gland developed in 10 of 15 strain A mice and in 2 of 4 strain C mice given injections of benzpyrene. This organ showed more rapid development of tumor than any other tissue in the body. The tumors were palpable in two to three and one-half months and attained diameters up to 2.5 cm. They were usually invasive and sometimes soft and cystic. The first stage of tumor development was a metaplasia of the glandular tissue into a stratified squamous epithelium, which then became malignant (fig. 2). The changes in the submaxillary gland parallel exactly the metaplastic and neoplastic changes which occurred in the prostate when benzpyrene was injected into that organ.<sup>19</sup> The submaxillary tumors were readily transplanted into animals of the same strain and to date have gone through five transplantations. In this connection it is of interest to note that squamous cell carcinoma of the skin is usually difficult to transplant. A spindle cell sarcoma was found five and one-half months after the injection of benzpyrene into the submaxillary gland of a mouse of the C<sub>3</sub>H strain, and 2 of 3 rats given injections of dibenzanthracene likewise showed spindle cell sarcomas in fifteen to sixteen months. It appears that the rapid rate of tumor formation in this gland is restricted to neoplasms of epithelial origin.

*Stomach and Duodenum*—In this experiment benzpyrene was injected into the submucosa of the pyloric region of the stomach in 5 rats and into the duodenum in 2. In 3 of the rats receiving injections in the stomach tumors developed, those receiving injections in the duodenum did not show development of tumors. The first tumor was noted at seven months, when a rat died of starvation. Autopsy revealed an obstruction in the lumen of the stomach, 3 by 3.5 cm in diameter. It was a myoma. The second tumor was found in a rat killed after thirteen months. It was a spindle cell sarcoma, 1 by 1.5 by 2 cm, in

18 Parsons, L. D. J. Path. & Bact. **43** 1, 1936. Burrows, H., and Cook, J. W. Am. J. Cancer **27** 267, 1936.

19 Moore, R. A., and Melchionna, R. H. Am. J. Cancer **30** 731, 1937.

the pyloric end of the stomach. The third tumor was a nodule 1 by 1 cm in the pyloric region of a rat which died after fifteen months. The tumor was a low grade adenocarcinoma which had invaded the muscularis. No metastases were found in any of the animals.

*Uterus*—Of 4 rats receiving injections of benzpyrene in the uterine wall, 1 subsequently showed a large myogenic sarcoma of the uterine horn.

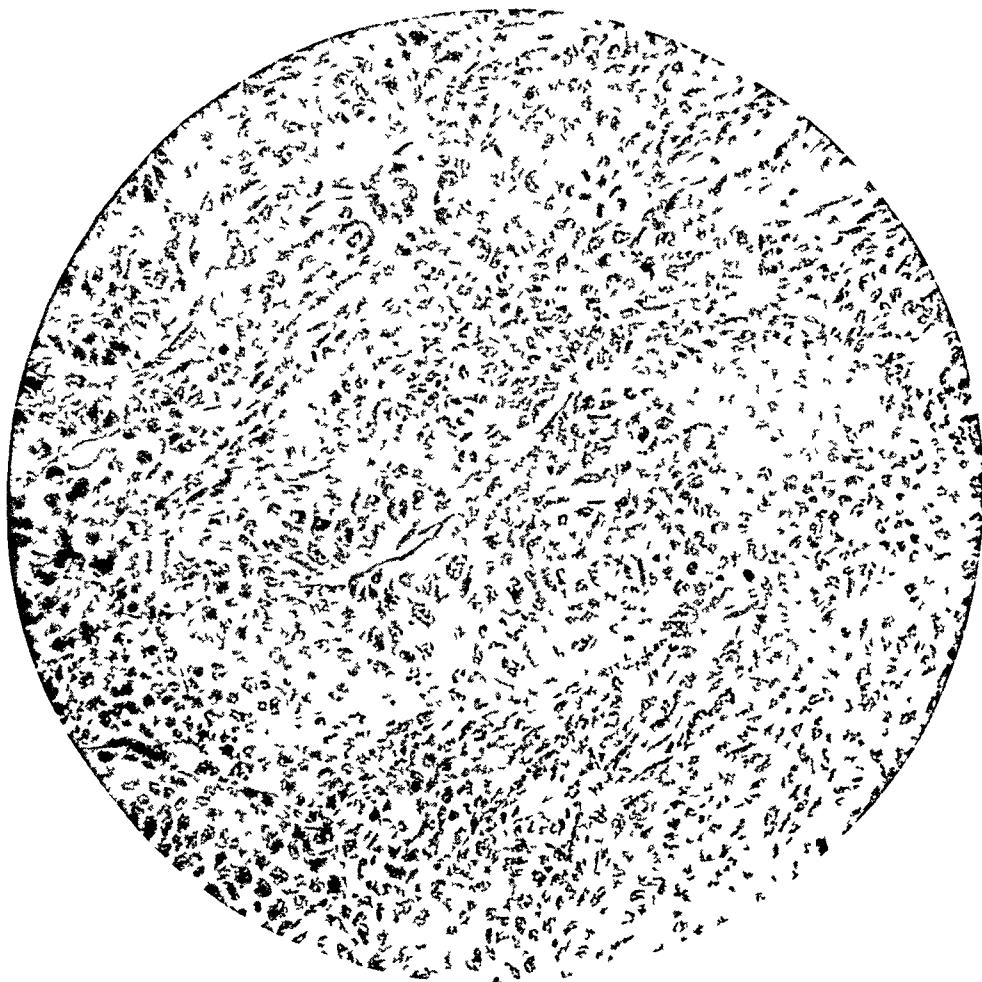


Fig. 2—Squamous cell carcinoma induced by an injection of benzpyrene into the submaxillary gland of a mouse.

(fig. 3) The animal died twelve months after the injection of benzpyrene.

*Spleen and Liver*—Of the animals receiving intrasplenic injections of benzpyrene or dibenzanthracene, 11 rats and 11 mice lived longer than five months. One only, a C<sub>3</sub>H mouse treated with dibenzanthracene, showed development of a tumor—a spindle cell sarcoma. There were 9 rats and 3 mice that received injections of dibenzanthracene.

in the liver. They survived for periods of six to seventeen months but failed to present tumors.

*Testis and Epididymus*—Large amounts of the hydrocarbons (table 2) were present in the testes of both rats and mice for periods longer than necessary for the production of tumors in other tissues. Nevertheless, no tumors developed. This confirms the result of Ilfeld<sup>20</sup> and

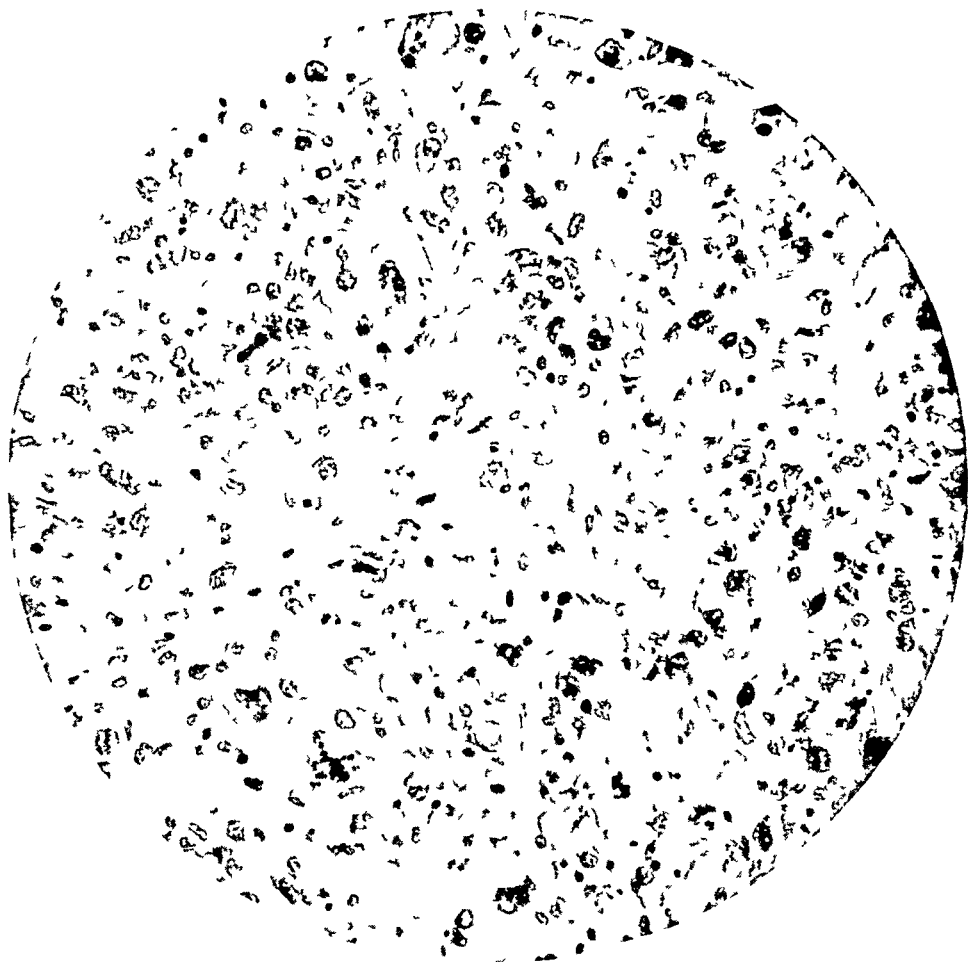


Fig. 3—Myogenic sarcoma induced by an injection of benzpyrene into the uterus of a rat.

of Tuchmann and Demay<sup>21</sup>. The local toxicity of the hydrocarbons produced marked parenchymal degeneration, but no other response to the chemicals appeared. Apparently both the testes and the epididymes of rats and mice are more refractory to the action of carcinogenic hydrocarbons than are other tissues.

20 Ilfeld, F. W. *Am. J. Cancer* **26** 743, 1936.

21 Tuchmann, H., and Demay, M. *Compt. rend. Soc. de biol.* **123** 686, 1936.

*Skin*—In 1 rat a raw area developed in the lumbar region of the back about fifteen months after the injection of dibenzanthracene into the spleen and into each testis, a total of 3 mg being administered. One month after the development of the raw area the animal was killed. The area was 2 by 2.5 cm in diameter and had firm raised edges. It was a spindle cell sarcoma which invaded the neighboring muscle tissue. It probably represented a tumor arising at an injured site in an animal loaded with a carcinogenic agent. A similar spontaneous tumor has never been found in our rat colony.

*Results of the Feeding of Wheat Germ Oil*—Both rats and mice on the wheat germ oil diets remained in good health. No neoplasms developed. On the diet containing 7 per cent wheat germ oil, 5 mice died within three months, while 10 survived for six to ten months, and 10 were still alive at fourteen months. Survival was much poorer when aminoazotoluene was added to the wheat germ oil, 7 died within four months and the remaining 18 died in six to eight months. The rats were killed after receiving 25 per cent wheat germ oil for four months. No abnormalities of any kind were noted, although Rowntree and associates<sup>12</sup> reported tumors occurring within six weeks. We were therefore unable to confirm the report that wheat germ oil causes peritoneal tumors.

#### COMMENT

It is evident that there is a marked difference in the response of various animal cells to carcinogenic agents, since tumors develop in some tissues much more readily than in others. The standard tissues for experimental production of tumors are the cutaneous and subcutaneous tissues. When benzpyrene was injected subcutaneously into 200 of our mice of the C strain, it took five to five and one-half months for 50 per cent of the animals to show development of tumors. When benzpyrene was applied to the skin in benzene solution, the time necessary for tumors to develop in 50 per cent of the animals was five and one-half months, both in 420 "C" mice and in 160 commercial mice. This is in line with the experience of Andervont,<sup>6</sup> who observed no essential difference in the incidence of subcutaneous tumors of strain A and C mice following injection of dibenzanthracene, although a considerable difference does exist among several strains. In our experiments with benzpyrene the per cental tumor incidence in the cutaneous and subcutaneous tissues was over 70 in all groups. In all other tissues tried except the submaxillary gland the per cental tumor incidence was less than 40. The rate of tumor production also varied in the various organs. All the submaxillary tumors of epithelial origin developed within three and one-half months, as compared with five and one-half months for

50 per cent of the cutaneous or subcutaneous tumors. In the other tissues tumors were produced more slowly if at all.

The reasons for this irregularity are not apparent. Conceivably one might associate rapid development of tumor with an adequate supply of blood, but this certainty is not the dominant factor, since the supply of blood to the skin and subcutaneous tissue is appreciably less than that to the liver and testis. Nor does there appear to be a connection between the rate of tumor formation and the rate of cellular proliferation in various tissues. The skin, which is proliferating, shows no more rapid development of tumors than subcutaneous tissue, in which cellular division is not so active. Furthermore, proliferation is extensive in both the bone marrow and the testis, in neither of which when they were exposed to benzpyrene did tumors develop. Finally, it does not appear that such differences can be explained on the basis of a difference in the quantity or character of the connective tissue present in various organs. However, it appears that certain cells within an organ are more susceptible to neoplastic transformation than other cells although all have equal contact with the carcinogenic agent. This difference is clearly illustrated by the rapid formation of epithelial tumors in the submaxillary gland whereas only one tumor of connective tissue origin occurred and that at a much slower rate. The same holds true in the case of the prostate, as squamous cell carcinoma developed in 75 per cent and sarcoma in 5 per cent of the prostates of rats given injections of benzpyrene.<sup>19</sup>

The ease of formation of benzpyrene tumors in any tissue does not appear to parallel the susceptibility of that tissue to spontaneous tumor formation. Spontaneous tumors of the submaxillary gland or of subcutaneous tissue are not particularly common either in man or in the lower animals, as might be expected from the rapid response of these tissues to benzpyrene. Nor does resistance to benzpyrene indicate resistance to spontaneous tumor formation, spontaneous tumor of the testis, duodenum or liver being by no means rare. Thus, while a satisfactory explanation may be lacking, the fact remains that the response of a given tissue to a carcinogenic agent is specific both for the cells and the type of carcinogenic agent. A given result obtained under one set of carcinogenic factors does not enable one to predict the response of that tissue to another set of carcinogenic factors.

#### SUMMARY

The following organs of mice or rats were given injections of 3,4 benzpyrene or 1,2,5,6-dibenzanthracene: submaxillary gland, spleen, liver, testis, epididymis, uterus, stomach (submucosa), duodenum (submucosa) and bone marrow. The tumors resulting were 12

squamous cell carcinomas and 3 spindle cell sarcomas of the submaxillary gland, 1 myoma, 1 spindle cell sarcoma and 1 adenocarcinoma of the stomach, 3 periosteal fibrosarcomas, 1 myogenic sarcoma of the uterus, and 1 spindle cell sarcoma of the spleen. The development of squamous cell carcinoma of the submaxillary gland was extremely rapid. The injection of the hydrocarbons into the bone marrow did not produce changes in the blood picture. No tumors were produced by the feeding of wheat germ oil.

# DISEASE OF THE LIVER IN HYPERTHYROIDISM

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The relationship between toxic disease of the thyroid gland and concurrent damage of the liver is one which has been little recognized and, until late years, commonly ignored. An extensive review of "the historical data concerning the hepato-thyroid relation" has recently been published by Boyce and McFetridge<sup>1</sup>. Paul, in 1865, was the first to mention the association of hepatic cirrhosis and toxic thyroid disease, although Eden, in 1906, seems to have been the first to realize the possibility of a causal relationship.

Although numerous individual cases have been reported and references to a probable relation made, it has been only since 1933 that any detailed studies of the pathologic changes in the liver have appeared in the literature, viz., those by Weller<sup>2</sup> (48 cases), 1933, Beaver and Pemberton<sup>3</sup> (107 cases), 1933, Rossle<sup>4</sup> (30 cases), 1933, Haban<sup>5</sup> (26 cases), 1933-1934, and Cameron and Karunaratne<sup>6</sup> (30 cases), 1935. The findings in these 241 cases are summarized by Cameron and Karunaratne<sup>6</sup>.

Various investigators have reported clinical evidence of hepatic dysfunction obtained by utilization of liver function tests. These include Maddock, Collier and Pedersen<sup>7</sup> (bromsulphthalein test), Huxthal<sup>8</sup> (blood cholesterol determination), Rowe<sup>9</sup> (various tests), Ragins<sup>10</sup> (Takata-A1a test) and Althausen and Wever<sup>11</sup> (galactose tolerance test). A recent editorial in *The Journal of the American Medical Association*

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From the Department of Pathology, University of Cincinnati, and Cincinnati General Hospital

- 1 Boyce, F F, and McFetridge, E M Arch Surg **37** 427, 1938
- 2 Weller, C V Ann Int Med **7** 543, 1933
- 3 Beaver, D C, and Pemberton, J D Ann Int Med **7** 687, 1933
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- 5 Haban, G Beitr z path Anat u z allg Path **92** 88, 1933
- 6 Cameron, G R, and Karunaratne, W A E J Path & Bact **41** 267, 1935
- 7 Maddock, W G, Collier, F A, and Pedersen, S West J Surg **44** 513, 1936
- 8 Huxthal, L M Arch Int Med **52** 86, 1933
- 9 Rowe, A W Endocrinology **12** 1, 1928
- 10 Ragins, A B J Lab & Clin Med **20** 902, 1935
- 11 Althausen, T L, and Wever, G K J Clin Investigation **16** 257, 1937

ciation<sup>12</sup> called attention to the work done by Bartels and by Boyce and McFetridge utilizing the Quick hippuric acid test for determining the amount of hepatic injury in thyrotoxic patients

There is by no means universal acceptance of the concept of hepatic insufficiency occurring as a result of hyperthyroidism Means<sup>13</sup> found scant evidence supporting such a concept and classified the hepatic injury as a complication rather than as a characteristic of the disease Mallory, quoted by Means,<sup>14</sup> suggested that the thyroid hormone itself does not injure the liver but that in certain cases hyperthyroidism may lead to hepatic dysfunction indirectly by increasing the vulnerability of that organ to noxious agents

Experimentally it has been repeatedly shown that the feeding of desiccated thyroid reduces the glycogen content of the liver in a large variety of animal species Farrant<sup>15</sup> demonstrated "fatty degeneration" of the liver in thyroid-fed rabbits and cats, while Gerlei<sup>16</sup> observed necrosis in the centers of liver lobules in rabbits five to seven days after administering lethal doses of thyroxin subcutaneously Hashimoto<sup>17</sup> produced parenchymatous changes in the liver and in the convoluted tubules of the kidneys of albino rats by feeding thyroid substance However, Youmans and Warfield<sup>18</sup> failed to note such changes in dogs given large amounts of thyroid extract, nor did liver function tests show any evidences of dysfunction

Mention should also be made of accumulating evidence that vitamin deficiency may play a part in hepatic insufficiency Ivy<sup>19</sup> expressed the belief that there is a definite relationship, direct or indirect, between the liver, vitamin B<sub>1</sub> and the vitamin B<sub>2</sub> complex, and the thyroid He quoted Drill<sup>20</sup> as showing that the administration of the vitamin B<sub>2</sub> complex protects the liver from the deglycogenizing effect of thyroxin

#### MATERIAL

All cases of well established clinical toxic thyroid disease in which necropsy was done at the Cincinnati General Hospital during the period from 1926 to 1938 were studied Cases were eliminated in which there were other, independent

12 Hippuric Acid Test as an Index of Hepatic Damage, editorial, J A M A **111** 1470, 1938

13 Means, J H Thyroid and Its Diseases, Philadelphia, J B Lippincott Company, 1937, p 319

14 Means,<sup>13</sup> p 292

15 Farrant, R Brit M J **2** 1363, 1913

16 Gerlei, F Ann d'anat path **10** 555 1933

17 Hashimoto, H Endocrinology **5** 579, 1921

18 Youmans, J B, and Warfield, L M Arch Int Med **37** 1, 1926

19 Ivy, A C Personal communication to the author

20 Ivy, A C Internat Abstr Surg **66** 4, 1938, in Surg, Gynec & Obst, January 1938 Drill, V A J Nutrition **14** 355, 1937



anatomic factors which might have produced hepatic pathologic changes or in which there was uncertainty about the clinical diagnosis. A total of 24 cases was collected (table 1).

A detailed study was made of representative microscopic sections in each case. When indicated, slides were restained or new sections were prepared from gross material preserved at necropsy.

For a careful comparative study of the pathologic observations in the liver in routine necropsy material, two series of cases were selected. The hepatic changes were studied in a group of 50 consecutive cases in which death from traumatic injuries occurred within forty-eight hours after the accident. Because of the possibility that a certain amount of hepatic change in cases of thyrotoxicosis might be secondary to passive venous congestion, analysis was made of a second group of 50 consecutive cases in which the cause or primary contributory cause of death was chronic rheumatic heart disease. Eliminated from both groups were all cases not falling within the age limits of the toxic thyroid series. No other exclusions were made.

A study of the pathologic changes in the liver in cases of vitamin B and vitamin C deficiency, occurring singly and combined, was also made to determine whether

TABLE 1—*Clinical Diagnoses Made in Twenty-Four Cases of Hyperthyroidism*

Clinical Diagnosis	Cases	Males	Females
Toxic nodular goiter	14	1	13
Toxic diffuse goiter	6	2	4
Adenomatous goiter with secondary hyperthyroidism	3		3
Adenocarcinoma of the thyroid	1		1
Totals	24	3	21

there were any changes common to this group and cases of toxic thyroid disease. Thirty-three such cases were collected, in this group the most frequent clinical diagnosis was pellagra.

#### CLINICAL FINDINGS

The group of 24 thyrotoxic patients comprised 3 males and 21 females. The preponderance of the latter was higher than is usually observed and was probably due to the relatively small total number of patients. There were 18 white and 6 Negro patients. The average age was 53 years, the youngest being 27 years old and the oldest being 76 years. Included in the group were 2 persons aged 76 and 74 years, respectively. The former had been operated on sixteen years before death (bilateral superior pole ligations were performed at that time) and had suffered a recurrence of symptoms during her last year of life. The latter had an enlarged thyroid for thirty-three years with symptoms of thyrotoxicosis for slightly more than a year preceding death. Three patients were admitted in thyroid crisis. Two of these were jaundiced.

Icterus has often been described as a clinical finding in toxic thyroid disease. In this series it was present in 5 persons. In 3 of these jaundice was well marked and in 2 evident only in the scleras. For the entire series of 24 patients the basal metabolic rates ranged from +18 to +73 per cent, the average being +41 per cent. The duration of symptoms averaged slightly less than two years. Iodine therapy was instituted in 67 per cent, the average duration of treatment being eleven days. No liver function tests were performed. In 2 recorded cases the

icteric index was +36 and +75. Two patients had marked pitting edema of the lower extremities and reversal of the albumin-globulin ratio. A history of alcoholism was obtained from 3 patients. One of these admitted moderate indulgence, the other 2 were definitely addicts. Diabetes mellitus was an associated condition in 2 patients. The Wassermann reactions of the blood of 3 patients were positive.

#### PATHOLOGIC OBSERVATIONS

A reduction of liver weight in fatal cases of hyperthyroidism has been mentioned by various authors. Beaver and Pemberton<sup>3</sup> stated that loss of weight of the liver was possibly the most important hepatic change which they found, and gave 1,316 Gm as the average weight in their series. In the present series of 24 cases of toxic thyroid disease the average weight of the liver was found to be 1,275 Gm (table 2). The average weight found in 50 cases of chronic rheumatic heart disease

TABLE 2—*Incidence of Common Pathologic Changes of the Liver in Various Reported Series of Fatal Cases of Hyperthyroidism*

Series	Cases	Average Weight of Liver, Gm	Percentage of Cases with Given Hepatic Change		
			Fatty Infiltration	Chronic Localized Interstitial Hepatitis (Including Cirrhosis)	Cirrhosis (All Stages)
Rossle (1933)*	30	1,225			
Haban (1933)*	26		23.0		38.5
Weller (1933)	48		"Frequently noted"	87.5	
Beaver and Pemberton (1933)	107	1,316	87.8	59.8	14.9
Cameron and Karunaratne (1935)	30	1,157	80.6		33.3
Present series	24	1,275	91.7	83.3	25.0

\* The series of Rossle and of Haban are not complete.

was 1,600 Gm, while in 50 cases of accidental death it was 1,570 Gm. It is true that loss of body weight is a constant finding in hyperthyroidism and that the weight of the liver should perhaps be computed as percentage of body weight. Nevertheless, just as loss of general weight is accepted as a clinical manifestation of the disease, loss of hepatic weight should be regarded as a pathologic change.

Fatty infiltration of varying degrees is commonly observed in the liver. Probably the two factors of greatest importance in causing this are intoxication and insufficient oxygenation. Canzanelli and Rapport<sup>21</sup> found that the consumption of oxygen by the guinea pig liver was depressed when thyroid tissue was incubated with it. This led them to suspect the presence of a substance in the thyroid inhibiting oxygenation.

<sup>21</sup> Canzanelli, A. and Rapport, D. *Endocrinology* **22**: 73, 1938.

Connor<sup>22</sup> expressed the belief that chronic severe fatty infiltration of the liver progresses in many cases to "fatty cirrhosis." With Chaikoff and Biskind<sup>23</sup> he recently demonstrated that cirrhosis develops following severe fatty infiltration in depancreatized diabetic dogs. Fatty infiltration occurred in all but 2 of my series of 24 cases of toxic thyroid disease. The picture was that commonly seen in fatty metamorphosis of the liver. Care was taken to use the same standards throughout, not only in judging this change but in judging all pathologic changes noted in the liver. Independent observations were made by another observer and the results carefully checked. The degree or intensity of the changes was specified by three descriptive terms—"mild," "moderate" and "marked" (table 3). Of the 50 cases of accidental death, a diagnosis of alcoholism was made in 4. Probably the incidence of alcoholism would have been much higher had it not been for the fact that a considerable proportion of the patients died before regaining

TABLE 3—*Incidence of Fatty Infiltration of the Liver in a Series of Cases of Hyperthyroidism and in Control Series*

Series	Cases	Number Showing Given Degree of Fatty Infiltration			
		Mild	Moderate	Marked	Total
Toxic thyroid disease	24	6	9	7	22
Chronic rheumatic heart disease	50	12	10	3	25
Accidental death	50	15	10	6	31

consciousness, so that no history was obtained. Fatty infiltration of the liver was observed in 62 per cent of the cases in this group. It was encountered in 50 per cent of the cases of chronic rheumatic heart disease and was almost invariably associated with severe chronic passive congestion. Attention should be drawn also to the differences in severity of this acute degenerative process in comparative studies. Moderate to markedly severe fatty changes were encountered in the liver in 67 per cent of the cases of toxic thyroid disease, while of this range changes were observed in only 26 per cent and 32 per cent of cases, respectively, of chronic rheumatic heart disease and accidental death.

While fatty infiltration of the liver may perhaps be considered an acute degenerative process, the presence of a low grade inflammatory reaction at the peripheries of liver lobules, associated often with patchy increase in fibrous connective tissue and with lymphocytic infiltration, is certainly indicative of a more chronic process. It is consistently found in its

22 Connor, C. L. J. A. M. A. **112** 387, 1939.  
23 Chaikoff, I. L., Connor, C. L., and Biskind, G. R. Am. J. Path. **14** 101, 1938.

most marked form in true diffuse cirrhosis of the liver. This form of chronic localized interstitial hepatitis was found in 83 per cent of the 24 cases reported here (table 4). The sole criterion for making the diagnosis was lymphocytic infiltration of the periportal areas. No attempt was made to estimate changes in fibrous connective tissue except where marked periportal fibrosis or definite cirrhosis was evident. Comparative studies showed this lesion to be present in 22 and 26 per cent of cases, respectively, of accidental death and chronic rheumatic heart disease. Furthermore, the degree of change was ordinarily far more intense in the cases of thyrotoxicosis than in the control groups.

The criteria used for the diagnosis of cirrhosis were degenerative changes leading to the disappearance of hepatic cells, a chronic inflammatory reaction occurring at the peripheries of the altered lobules, and regeneration of an imperfect type, such changes being generally distributed in the liver. Cirrhosis was found in 6 of the 24 cases, it was advanced in 2 and of moderate degree in 4. In only 1 instance was it

TABLE 4—*Incidence of Chronic Localized Interstitial Hepatitis in a Series of Cases of Hyperthyroidism and in Control Series*

Series	Cases	Number Showing Given Degree of Chronic Localized Interstitial Hepatitis			
		Mild	Moderate	Marked	Total
Toxic thyroid disease	24	10	6	4	20
Chronic rheumatic heart disease	50	8	4	1	13
Accidental death	50	7	3	1	11

found in the accidental death series and in no cases in the chronic rheumatic heart disease series. During the years 1935 and 1936, in 1,431 consecutive necropsies the diagnosis was made sixty-nine times, an incidence of 4.7 per cent. In the thyroid series there were 3 cases in which periportal lymphocytic infiltration was associated with patchy fibrosis. The possibility exists that this is an early stage in the development of cirrhosis.

It is difficult to evaluate the incidence of cirrhosis in this as compared with other reported series because of the dissimilarities in diagnostic criteria. Most observers have classified increased periportal fibrosis with the more specific, easily recognized cirrhoses and have called it by various terms, such as "insular cirrhosis," "early chronic atrophic cirrhosis" and "patchy chronic parenchymatous interlobular hepatitis" (table 2). Weller expressed the belief that cirrhosis of the liver in hyperthyroidism occurs only as an advanced stage of chronic interlobular hepatitis. Hahan, quoted by Bartels,<sup>24</sup> suggested the name "cirrhosis

Basedowiana" for the condition of those livers found in fatal cases of toxic thyroid disease which show any chronic lesions of a cirrhotic type

Hepatic lesions secondary to chronic venous stasis were observed in 9 of the cases of thyrotoxicosis. The lesions most frequently encountered were dilatation of venous sinusoids, atrophy of liver cords and varying degrees of necrosis of the central zones. A careful check on these changes was provided by the series of fatal cases of chronic rheumatic heart disease, in which well marked hepatic lesions secondary to chronic passive congestion were found in 80 per cent of the cases. Well marked hepatic fibrosis was observed in 9 of the 50 cases of chronic rheumatic heart disease, however, this increase in connective tissue in all cases originated from the centers of the lobules instead of appearing in the periportal regions and was usually most marked in the subcapsular lobules. There was no evidence of associated chronic interstitial

TABLE 5—Incidence of Common Hepatic Lesions in a Series of Cases of Deficiency of Vitamin B<sub>1</sub>, the Vitamin B<sub>2</sub> Complex and Vitamin C as Compared with a Series of Cases of Hyperthyroidism

Series	Cases	Number Showing Given Degree of Fatty Infiltration				Number Showing Given Degree of Chronic Interstitial Hepatitis				Cirrho sis
		Mild	Moder ate	Marked	Total	Mild	Moder ate	Marked	Total	
Vitamin B and C defi ciencies	33	14	8	11	33	14	6	1	21	0
Toxic thyroid disease	24	6	9	7	22	10	6	4	20	6

hepatitis. The lesion observed was that sometimes referred to as cardiac cirrhosis.

Microscopic study was made of the livers of 33 patients whose death was caused directly or indirectly by deficiency of vitamin C, vitamin B or the vitamin B<sub>2</sub> complex. There were histories of alcoholism in 14 of these patients. Although fatty infiltration was evident in every liver, the degree of involvement was more than moderate or marked in only 57 per cent as contrasted with an incidence of 67 per cent in the toxic thyroid series. Well marked chronic interstitial hepatitis was found in only 20 per cent and cirrhosis in none of these livers (table 5).

Microscopic sections of the thyroid gland were reviewed in every case. There was marked variation in the pathologic changes not only between sections from different glands but also between sections from the same gland. Since iodine therapy had been instituted in the majority of the cases, varying degrees of involution were seen. In 19 of the 24 cases there was definite evidence of epithelial hyperplasia. Associated with this proliferation of epithelial cells there were observed varying amounts of colloid, usually poorly stained and vacuolated. An increased

number of newly formed blood vessels and lymphocytic infiltration in the interacinar tissue were also noted in almost every one of these 19 cases. In 4 of the 24 cases marked involutionary changes were present, with occasional epithelial spurs extending into the acini. The colloid was well stained and the epithelium lining the acini flattened. In each of these 4 cases, however, lymphocytic hyperplasia in the interstitial tissue was a constant finding. The involutionary changes were believed to be due either to intensive iodine therapy or to a remission of the hyperthyroidism. In 1 of the 24 cases a typical picture of adenocarcinoma was observed.

#### ASSOCIATED CHANGES OBSERVED AT NECROPSY

The possibility that pathologic changes may occur in the kidneys secondary to degenerative hepatic changes has been advanced by numerous observers. The term "hepatorenal syndrome" has frequently been applied to the association, and various theories have been advanced to explain clinically evident renal insufficiency or abnormal urinary findings in the presence of hepatic disease. Many investigators who have produced hepatic lesions by feeding thyroid substance have reported associated renal changes, which they have ascribed solely to the induced hyperthyroidism. Thus Farrant<sup>15</sup> describes, in addition to "fatty degeneration" of the liver in thyroid-fed cats and rabbits, changes in the kidneys suggestive of "tubular nephritis." Both Hashimoto<sup>17</sup> and Goodpasture<sup>25</sup> described parenchymatous changes in the convoluted tubules of the kidneys as well as in the livers of thyroid-fed albino rats. However, Bartels<sup>26</sup> found no disturbance in renal function as tested by the urea clearance test in 25 cases of hyperthyroidism.

In the present study, marked degenerative changes of the renal convoluted tubules were noted in association with advanced cirrhosis of the liver in 1 case. Extensive cytoplasmic degeneration of the tubular epithelium was evident, and with the scarlet red staining technic in combination with study under the polarizing microscope it was possible to demonstrate large amounts of doubly refractile lipid in the epithelial cells. There were no glomerular changes to support a diagnosis of glomerulonephritis. In 4 other cases tubular degenerative changes of lesser degree were noted, in 1 of which they were associated with cirrhosis of the liver. Vacuolization of the epithelium of the loops of Henle was seen in the 2 cases complicated by diabetes mellitus.

Focal degeneration of the myocardium, often described in fatal cases of hyperthyroidism, was not noted in any case. Changes in the adrenal

25 Goodpasture, E. W. *J. A. M. A.* **76** 1545, 1921, *J. Exper. Med.* **34** 407, 1921.

26 Bartels, E. C. *New York State J. Med.* **39** 117, 1939.

bodies consisted of cortical nodular hyperplasia in 3 cases, so-called cortical adenoma in 2 and early adenocarcinoma in 1. Hyperplasia of the thymus was observed in 6 cases. Leiomyoma of the uterus was found in 12 of the 20 females in the group.

#### COMMENT

A great deal of attention has been paid to clinical observations of hepatic insufficiency in thyrotoxic patients. Comparatively little note has been made of the pathologic changes in the liver which might produce this dysfunction. Unfortunately, no liver function tests were done on the 24 patients in this study. However, icterus occurred in 5, well marked in 2 and mild in 3. In a patient with an icteric index of +36 the most important etiologic factor was probably extensive passive congestion, although fatty infiltration also was observed. In 3 patients jaundice was associated with cirrhosis of the liver. In the fifth patient, a young man with a clinical history of only three months' duration, who died in thyroid crisis, icterus was confined to the scleras, and the only significant hepatic change was extensive fatty infiltration.

It has been suggested by various investigators, Dinsmore<sup>27</sup> and Lahey<sup>28</sup> among others, that delirium and disorientation after thyroidectomy and even death associated with hyperthyroidism are chiefly due to hepatic failure. Certainly the almost invariable occurrence of pathologic changes in the liver in the 24 cases presented here is strong presumptive evidence of such a possibility. This is the more convincing since severe hepatic lesions, with the exception of fatty infiltration, were negligible in the control group of 50 cases in which death resulted from traumatic injuries. Except for changes secondary to chronic passive congestion, extensive alterations were found with relative infrequency also in the control group of 50 cases in which death was due to chronic rheumatic heart disease. In each of the 3 cases of hyperthyroidism in which crisis was a clinical manifestation there was moderate fatty infiltration of the liver. One of these cases showed associated chronic localized interstitial hepatitis, while another was complicated by well marked cirrhosis of the liver.

The possibility exists that too much importance has been placed on the constant finding of fatty infiltration of the liver in fatal cases of thyrotoxicosis, since such fatty change commonly occurs in many conditions other than hyperthyroidism. However, the high percentage of association together with the severity of the changes makes it impossible to ignore them. In the majority of cases a combination of several hepatic pathologic changes was noted. Probably the lesion of greatest signifi-

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<sup>27</sup> Dinsmore, R. S. J. A. M. A. **109** 179, 1937.

<sup>28</sup> Lahey, F. H. New England J. Med. **213** 475, 1935.

cance was chronic interstitial hepatitis, sometimes associated with periportal fibrosis or cirrhosis. While lymphocytic infiltration at the peripheries of liver lobules without known cause is occasionally encountered in routine necropsies, the degree of involvement is usually slight.

No lesions were observed similar to those reported in the livers of animals suffering from induced hyperthyroidism. Although central necrosis of liver lobules was not infrequently encountered, in every case chronic passive congestion could be demonstrated as the etiologic factor. On the other hand, congestion appeared to play no part in the production of chronic hepatitis or cirrhosis. Experimental evidence that administration of excessive amounts of thyroid results in depletion of the glycogen in the liver and that a good store of glycogen exerts a protective influence against poisons such as chloroform has suggested the hypothesis that the hepatic lesions in hyperthyroidism are due to exhaustion of hepatic glycogen. Cameron and Karunaratne<sup>6</sup> found that the minimal toxic dose of carbon tetrachloride for thyroid-fed albino rats remained the same as for normal control rats. Clinically, however, Bartels<sup>24</sup> recently demonstrated that the use of a diet high in carbohydrate improved liver function, as indicated by the excretion of increased amounts of hippuric acid.

An attempt was made to correlate clinical and pathologic observations, but the series of cases was too small to allow complete conclusions to be drawn. Beaver and Pemberton<sup>7</sup> expressed the belief that the common factor in the evolution of hepatic lesions appears to be related to the severity of the syndrome of exophthalmic goiter. In the present series, the average duration of symptoms in 6 cases of thyrotoxicosis associated with cirrhosis of the liver was two and a half years, if these 6 cases are excluded, the average duration of symptoms was slightly less than two years. Lymphocytic infiltration in the periportal regions was noted in all cases in which the duration of symptoms was more than one year but in only 8 of the 12 cases in which the history was one year or less.

Two patients in whom thyroid disease was complicated by diabetes mellitus presented severe fatty infiltration of the liver. In one of these cirrhosis was also present. Cirrhosis of the liver was present in both patients for whom a diagnosis of chronic alcoholism had been made clinically in addition to that of hyperthyroidism. The role that alcohol plays in the production of cirrhosis has been a source of controversy for many years among physiologists and experimental pathologists. The majority of investigators have been opposed to the concept of alcohol as a direct cause of cirrhosis. Recently Hall and Morgan<sup>29</sup> pointed

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29 Hall, E. M., and Morgan, W. A. *Arch. Path.* **27**: 674, 1939.



out that accumulating evidence indicates that for man, at least, the body and more especially the liver must be in a state of altered metabolism before cirrhosis will result from heavy drinking of alcoholic liquor. Possibly the hepatic lesions in these 2 patients might not have progressed to cirrhosis in the absence of heavy drinking. The liver of another patient, who gave a history of moderate indulgence, presented moderate fatty infiltration and mild chronic interstitial hepatitis.

#### SUMMARY

It is believed that the results of this study indicate a relationship between toxic disease of the thyroid gland and hepatic damage. The lesions of the liver observed in 24 fatal cases of toxic thyroid disease were loss of liver weight, fatty infiltration, cirrhosis and lymphocytic infiltration in the periportal regions, often associated with patchy fibrosis. These hepatic changes appeared with greater frequency and severity in this series of 24 cases than in a carefully studied group of 100 control cases.

The average weight of the liver in the thyrotoxic group was 1,275 Gm, as compared with 1,582 Gm in the control group. Fatty infiltration was found in 92 per cent of the cases, while in only 56 per cent of the control group was there any evidence of this change. Evidence of a chronic inflammatory reaction was found in 83 per cent of cases of toxic thyroid disease whereas the incidence in the control series was only 24 per cent. The incidence of cirrhosis of the liver was 25 per cent in cases of hyperthyroidism and 4.7 per cent in 1,431 consecutive routine necropsies.

The hepatic lesions found in association with thyrotoxicosis cannot be said to result from passive venous congestion. Their production does not appear to be related to deficiencies of vitamins B and C. There is a possibility that the degenerative lesions of the renal tubules which occur in patients suffering from hyperthyroidism may be due directly to the thyrotoxicosis or secondarily to the hepatic changes.

Despite the limited number of cases in the present study it seems evident that more chronic lesions are found in the livers of patients with longer histories of toxic thyroid disease. The character of the pathologic changes in the liver in fatal cases of thyrotoxicosis appears sufficient to explain the clinical evidences of hepatic insufficiency.

# CHEMISTRY OF ATHEROSCLEROSIS

## I LIPID AND CALCIUM CONTENT OF THE INTIMA AND OF THE MEDIA OF THE AORTA WITH AND WITHOUT ATHEROSCLEROSIS

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Numerous studies have been made in the attempt to determine the cause and importance of the lipid deposits in the lining of the aorta and of the large arteries. Most of them have been morphologic rather than chemical. Neither method has provided all the information necessary to interpret the conditions. Some authors considered the lipid deposits incidental in the evolution of atherosclerosis, others regarded them as integral factors. The first view <sup>1</sup> emphasized focal injuries of the media, either anatomic (Thoma, Faber) or functional (Lange, Staemmler), as the initial changes. It interpreted the intimal hyperplasia as compensatory and the fatty and other retrogressive changes as secondary or, at most, of limited importance. According to the second view <sup>1</sup> (Marchand, Lubarsch, Aschoff, Anitschkow, <sup>2</sup> Leary,<sup>3</sup> and others), the essential factor is the penetration of the plasma lipids into the intima. Mechanical conditions favor plasma infiltration into the regions where subsequent degenerative changes and tissue reactions occur.

Wells <sup>4</sup> reviewed the early chemical studies. These analyses demonstrated an increase in the lipid content of the aorta with advancing age and severity of atherosclerosis. The lipid extracts from atherosclerotic aortas were reported to contain cholesterol, "lecithin" and fatty acids. In 1926 Schonheimer <sup>5</sup> in a systematic chemical analysis of atherosclerotic aortas observed an increase of the total lipids and a variation in the proportions of the various lipid constituents with increasing

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1. Beitzke, H. Virchows Arch f. path. Anat. **267** 625, 1928.

2. Anitschkow, N. Virchows Arch f. path. Anat. **249** 73, 1925.

3. Leary, T. Arch. Path. **17** 453, 1934.

4. Wells, H. G., in Cowdry, E. V. Arteriosclerosis, New York, The Macmillan Company, 1933.

5. Schonheimer, R. Ztschr. f. physiol. Chem. **160** 61, 1926.

severity of atherosclerosis The cholesterol esters varied from about 10 per cent of the total lipids in the aortas of youths to 60 per cent in those with atherosclerosis The average amount of free cholesterol remained constant Schonheimer<sup>6</sup> later reported an average of 3 per cent for the phospholipids and a progressive increase with age and degree of atherosclerosis His phospholipid values were low, probably because of the method of extraction He also found undetermined small amounts of fatty acids, "protagon" and a substance similar to "oxycholesterol" Neutral fats were not identified Page and Menschick<sup>7</sup> found a substance having absorption bands at 238 and 320 millimicrons in the cholesterol fraction of calcified aortas These absorption bands resemble those of cholestenone The significance of this observation is uncertain Cholesterol from other viscera did not contain this light-absorbing component Schonheimer<sup>8</sup> confirmed its presence in extracts of atherosclerotic aortas He was unable to detect ergosterol by spectroscopic examination, or any trace of vitamin D by biologic assay, in calcified aortic tissues

Kimmelstiel's<sup>9</sup> systematic analysis of human aortas for cholesterol, phosphatides and galactosides demonstrated a proportional increase of all fractions until atheromas formed Then the relationships changed and the cholesterol increased rapidly in comparison with the other fractions The galactoside content reported by Kimmelstiel amounted to 1 to 1.5 per cent of the dry weight of the aorta Lehnher,<sup>10</sup> in a comparative study of aortas in diabetes and nondiabetic conditions, observed that arteriosclerosis in the aorta is associated with (1) increased amounts of cholesterol, phospholipids and fatty acids, (2) an increased proportion of cholesterol in the total lipids, and (3) increased amounts of calcium and phosphorus with a diminished Ca/P ratio The lipid deposits and other changes in diabetes were similar but more marked Rosenthal's<sup>11</sup> analysis of 500 aortas for "fat" content confirms the previous reports that atherosclerosis is associated with an increased lipid content of the tissue He made no attempt to determine the individual lipids The analyses mentioned thus far were made on extracts of entire aortas

Meeker and Jobling<sup>12</sup> emphasized the local distribution of the lesions in atherosclerosis and stated that an aorta may have several stages of the disease They correlated the lipid analyses of individual lesions with

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6 Schonheimer, R. *Ztschr f physiol Chem* **177** 143, 1928

7 Page, I. H., and Menschick, W. *Naturwissenschaften* **18** 585, 1930

8 Schonheimer, R. *Ztschr f physiol Chem* **211** 65, 1932

9 Kimmelstiel, P. *Virchows Arch f path Anat* **282** 402, 1931

10 Lehnher, E. R. *New England J Med* **208** 1307, 1933

11 Rosenthal, S. R. *Arch Path* **18** 473 and 606, 1934

12 Meeker, D. R., and Jobling, J. W. *Arch Path* **18** 252, 1934

the character of the atherosclerotic process. With age, an increase in lipid content, especially of total cholesterol, and a progressive increase in the ratio of combined to free cholesterol were noted in normal tissues. In pathologic tissues the ratio increased, then decreased with the appearance of necrosis and calcification. The proportion of phospholipids remained constant. Zeek<sup>13</sup> reported the same findings.

The purpose of this study is to determine the variations of the lipid constituents and of the calcium content of the intima and of the media of the aorta with increasing age and with increasing severity of atherosclerosis. The aortas used for chemical analysis were obtained during routine postmortem examinations at St. Luke's Hospital. The adventitia and associated fat were stripped off without difficulty and discarded. The more difficult separation of the intima from the media was accomplished by placing the aorta with the intima down on a flat surface. After a diagonal cut had been made with a razor blade at one corner, the separation was started with the thumb nail, and the two coats were pulled apart. The intima was thin, translucent and without the fibrous character of the media. The fat deposits generally were limited to the intima. When the coats were separated, there was no trace on the media of the lesion in the intima. In marked atherosclerosis with extensive ulceration and necrosis, the media also was involved. The separation of the intima from the media was especially easy in aortas of advanced age.

#### ANALYSIS

*Extractions*—Representative portions of the media from the thoracic and abdominal portions of the aorta were weighed in a stoppered bottle, then torn into strips  $\frac{1}{8}$  inch (0.32 cm) wide, cut into fine pieces and transferred to a fat-free paper extraction thimble. The tissues were extracted continuously with boiling redistilled 95 per cent alcohol for eight to twelve hours. The thimble was washed several times with warm ether, the ether washings were added to the alcohol extract. Further extraction with alcohol and ether demonstrated only small traces of residual lipid. The alcohol extract and the ether washings were evaporated to dryness in a water bath at 60 to 70 C. The oily residue was taken up in small amounts of warm purified petroleum benzine (petroleum ether) and the liquid filtered through a fat-free paper into a 50 cc volumetric flask. The volume was made up to 50 cc. Portions of this extract were used for the determinations of lipid.

*Determinations*—(a) Nonlipid residue. The extraction thimble was dried to constant weight in an oven at 110 C. The dry weight of the tissues was determined by adding the weights of the lipid-free residue and the total lipids.

(b) Total lipid. A 10 cc aliquot of the petroleum ether extract was pipetted into a tared porcelain crucible and placed in an incubator until the solvent had evaporated. The crucible was dried to constant weight in a vacuum desiccator.

(c) Ash content. The crucible containing the lipid residue was ignited to constant weight with a Meker burner. A few drops of nitric acid were used to remove the last traces of carbon.

(d) *Cholesterol* A portion of the petroleum ether extract was diluted with a 50 per cent mixture of alcohol and acetone until 2 cc contained between 0.1 and 0.2 mg of cholesterol. The free and total cholesterol were determined by a slight modification of the Schonheimer and Sperry<sup>14</sup> method. The color readings were made with the Sanford-Sheard<sup>15</sup> "photometer." Duplicate analyses checked within 5 per cent.

(e) *Phosphatide* Five cubic centimeter portions of the petroleum ether extract were pipetted into graduated 15 cc centrifuge tubes and the solvent was evaporated to 1 cc by immersing the tubes in hot water a few minutes. Eight cubic centimeters of acetone was added and the phosphatides precipitated by the Bloor<sup>16</sup> method. The ether-soluble fraction (lecithin plus cephalin) was separated from the ether-insoluble fraction according to Kirk<sup>17</sup>. The phosphorus was determined in each fraction by the Fiske and Subbarow<sup>18</sup> method except that the color readings were made photoelectrically.

(f) *Galactoside* Hydrolysis was carried out on 5 cc aliquots of the extract by Kirk's<sup>19</sup> method and galactose determined by the ceric sulfate titration method of Miller and Van Slyke<sup>20</sup>. As the reducing power of galactose is not the same as that of dextrose, the ceric sulfate was standardized against the former sugar. The reducing power of galactose was 80 per cent of dextrose, a result in agreement with the 83 per cent reported by Giragossintz, Davidson and Kirk<sup>21</sup>. Strict proportionality was observed over wide ranges of concentration.

(g) *Fatty acids* No direct method exists, unfortunately, for the determination of free fatty acids in lipid extracts. As free fatty acids are only a small percentage of the total lipid, a large error is introduced when the calculation is made by difference between the total and the phospholipid and cholesterol fatty acids. The percentage of residual lipid is probably a fair index of the fatty acid content, since Schonheimer<sup>8</sup> showed the absence of glycerides in significant quantities.

(h) *Calcium* A portion of the lipid-free residue was weighed in a porcelain crucible, then ashed. Several treatments with nitric acid oxidized traces of the organic matter. The ash was determined, the residue treated with a little hydrochloric acid and diluted to a definite volume, and the calcium in an aliquot determined by the Clark and Collip<sup>22</sup> method.

## RESULTS

*Media*—The variations in the lipid constituents of the media with advancing age are given in table 1. Although all these tissues appeared normal in the gross, microscopic examination disclosed that the media of the aged tissues, especially those with large plaques, had lipid deposits

14 Schonheimer, R, and Sperry, W M J Biol Chem **106** 745, 1934

15 Sanford, A H, and Sheard, C J Lab & Clin Med **15** 483, 1930

16 Bloor, W R J Biol Chem **82** 273, 1929

17 Kirk, E J Biol Chem **123** 623, 1938

18 Fiske, C H, and Subbarow, Y J Biol Chem **66** 375, 1925

19 Kirk, E J Biol Chem **123** 613, 1938

20 Miller, B F, and Van Slyke, D D J Biol Chem **114** 583, 1936

21 Giragossintz, G, Davidson, C, and Kirk, P L Mikrokhemie **21** 21, 1937

22 Clark, E P, and Collip, J B J Biol Chem **63** 461, 1925

Despite individual variations, there is a definite increase in the total lipid content of the media with advancing age. The increase is general for all lipids studied except the lecithin-cephalin fraction. The trends can be seen better in table 2, where the mediae have been divided into three age groups. The free cholesterol in the youngest age group is about

TABLE 1—*Variation in Composition of Media with Age*

No	Age	Moisture*	Lipid Extract†	Cholesterol†			Phosphatide†			Galactoside†	Nonlipid Residue	
				Free	Total	Ester	Ether Soluble	Ether Insoluble	Total		Ash†	Calcium†
1	12	66.7	5.94	0.66	0.94	0.47			1.64	0.85		
2	22	74.5	6.29	1.22	1.54	0.54	2.96	0	2.96	0.50	2.12	0.21
3	23	57.4	4.08	0.89	1.70	1.37	1.64	0.32	1.96	0.26		
4	27	53.0	6.08	0.90	1.90	1.69	1.57	0.11	1.68	0.60	1.92	0.40
5	28	72.5	7.50	1.45	3.38	3.26	2.06	0.41	2.47	0.33	1.83	0.19
6	38	68.4	6.30	1.01	1.14	0.22	1.92	0.36	2.28	0.67	2.56	0.51
7	43	67.5	9.55	1.77	3.19	2.40	2.06	0.67	2.73	0.46	4.71	1.23
8	45	69.0	9.18	1.32	1.76	0.74	2.27	1.35	3.62	1.01	3.05	0.70
9	49	75.4	9.71	1.41	2.08	1.13	1.00	1.26	2.26	2.12	5.75	1.30
10	49	72.5	5.77	1.16	1.84	1.15	1.46	0.33	1.79	0.99	4.38	1.10
11	51	74.3	7.82	1.68	3.32	2.78	1.99	0.33	2.32	0.76		
12	52	69.5	7.75	1.21	2.34	1.90	0.80	1.42	2.22	0.90	4.32	1.15
13	53	71.8	6.53	1.05	1.44	0.66			2.15	0.93		
14	53	71.4	8.08	1.68	2.33	1.10	0.96	3.18	4.14	1.00	4.69	1.13
15	58	74.8	10.40	1.32	2.88	2.63	0.20	2.49	2.69	1.35	7.67	
16	65	71.3	10.40	2.49	3.70	2.04			3.38	0.71	9.52	3.12
17	64	74.9	8.22	1.50	2.82	2.22						
18	67	66.1	10.60	1.62	3.74	3.57	1.62	0.89	2.51	1.56		
19	67	71.7	11.00	1.65	3.71	3.47	0.79	2.26	3.05	0.90		2.57
20	68	72.5	9.72	1.85	3.11	2.12	2.16	0	2.16	0.68	4.69	1.36
21	71	67.4	10.90	2.42	5.40	5.02	0.78	2.42	3.20	0.43	11.30	3.50
22	72	74.2	11.00	1.75	4.43	4.52	1.56	1.89	3.45	1.07	7.58	2.26
23	75	66.3	9.10	1.76	3.56	3.03	1.27	0.76	2.03	0.50	8.35	2.38
24	78	70.2	11.80	1.72	3.89	3.66	0.53	2.52	3.05	1.42	9.28	2.94
25	84	68.7	13.00	1.98	4.00	3.40	1.62	1.74	3.36	1.24	7.08	2.22

\* The content is given as percentage of wet tissue

† The content is given as percentage of dry tissue

TABLE 2—*Variation in Composition of Media by Age Groups*

Age Group	Moisture*	Lipid Extract†	Cholesterol†			Phosphatide†			Galactoside†	Nonlipid Residue	
			Free	Total	Ester	Ether Soluble	Ether Insoluble	Total		Ash†	Calcium†
0-40	68.4	6.42	1.05	1.78	1.24	2.13	0.22	2.21	0.59	2.11	0.33
41-60	71.8	8.31	1.36	2.23	1.44	1.25	1.53	2.70	1.09	4.90	1.10
61-84	70.3	10.57	1.87	3.84	3.30	1.29	1.59	2.91	0.95	8.25	2.57

\* The average content is given as percentage of wet tissue

† The average content is given as percentage of dry tissue

1 per cent of the dry weight of the media. The cholesterol ester is slightly higher, the ratio being about 1.2. In the middle age group both values increase slightly, but the ratio 1.1 remains about the same. In the third age group the values for free and combined cholesterol increase greatly, the former by about 40 per cent, the latter by 130 per cent. The ratio thus increases markedly to about 1.8.

The phosphatides in the mediae of group 1 are 2.21 per cent of the dry weight with the lecithin-cephalin proportion about 90 per cent of the total. Group 2 has an increase of 22 per cent of total phosphatide over group 1, due to the increase (600 per cent) in the sphingomyelin (ether-insoluble fraction), which greatly exceeds the decrease in the ether-soluble phosphatide of 41 per cent. In both phosphatide fractions there is a further slight increase in the highest age group, but the proportions remain essentially the same. The significance of these phosphatide values is unknown. The galactoside values parallel those of sphingomyelin. Too much significance should not be given these galactoside values, however, because of the inaccuracies inherent in the method of analysis. Though somewhat lower than those reported previously,<sup>20</sup> they are probably closer to the actual values.<sup>19</sup>

The percentage of unidentified lipid material increases slightly with age. The exact composition of this fraction is unknown, but a large proportion probably is free fatty acids.

The lipid values of the first two age groups undoubtedly represent values for normal tissue. Histologically, stainable lipid is absent. The mediae of the highest age group have some deposits microscopically, grossly, there was no evidence of such deposits. These lipids appeared microscopically as diffuse bands lying between, but not involving, the elastic fibers. Such medial involvement appeared only under necrotic intimal lesions, especially in the abdominal portion, and was confined to the layers adjacent to the intima.

Of the nonlipid constituents, the moisture content remained relatively constant. Individual variations were great probably because of evaporation during preparation. There was no marked trend with age. The calcium content of the media, low in young aortas, advanced regularly with age to 2.57 per cent of the dry weight in the oldest. This increase in calcium was without demonstrable microscopic change. The calcium content in general paralleled the total ash content.

*Intima*—The intimas chosen for analysis were free from gross lipid deposits. Microscopically, however, there were occasional foam cells, but the small number of these in comparison with the total tissue had no great significance. The large amount of lipid in the intima as compared with the media of the same aorta was remarkable. The calcium content of the intima was low in comparison with that of the media.

*Summary*—The results indicate that an accumulation of lipids, including both cholesterol and phospholipids, occurs in the aging process of the aorta. The increase is most marked in the cholesterol esters and sphingomyelin. There is no definite correlation between these increases in the media and the type of lesion in the intima.

The increase of calcium in the media with age seems to indicate a normal physiologic process without causal relation to, and probably not significant in, calcification of the intima. This appears clearly in a comparison of the calcium content of the media with the condition in the corresponding intima. For example, in the middle age group there are six media tissues, nos 7, 8, 9, 10, 12 and 14, with about the same calcium content. In two of these, 8 and 12, there were scarcely any changes of the intima, in nos 7, 10 and 12 there was moderate atherosclerosis, in no 9 there was severe atherosclerosis with extensive calcification. The lack of correlation between intimal and medial calcification is even more obvious in the highest age group. Nos 21 and 25 contained the lowest amounts of calcium, the corresponding intimas had marked calcification. No 16, very high in calcium, had a practically normal intima without calcification and with only slight fatty changes.

TABLE 3—*Variation in Composition of Intima with Age*

No	Age	Mois- ture*	Lipid Extract†	Cholesterol†			Phosphatide†			Galac- toside†	Nonlipid Residue	
				Free	Total	Ester	Ether Sol- uble	Ether Insol- uble	Total		Ash†	Cal- cium†
6	38	70.9	9.44				1.44	0	1.44	1.66	1.61	0
10	49	75.8	13.6	1.83	3.01	1.99	2.56	0.33	2.92	1.59	2.06	0.26
12	52	61.8	18.7	3.58	8.40	8.12	0.53	2.94	3.47	0.72	2.37	0.47
14	53	76.1	14.5	2.37	7.70	8.98			3.11	0.55	1.80	0.20
17	64	73.2	15.7	1.27	4.58	5.57	1.99	0.32	2.31	0.37	2.13	0.18
Average		71.6	14.4	2.26	5.92	6.16	1.63	0.90	2.65	0.98	1.90	0.23

\* The content is given as percentage of wet tissue

† The content is given as percentage of dry tissue

Though not enough normal intimas have been analyzed to permit generalizations as to the variation of lipid with age, intimal tissue has a relatively high lipid content and a low calcium content as compared with media.

The water content of media, in spite of large individual variation, has no significant variation with age. This observation does not support the hypothesis of Wells<sup>1</sup> that arteriosclerosis arises from an inability to hold the constituents in solution as a result of the dehydration of the elastic tissue with age.

The results recorded in the preceding paragraphs disclose with advancing age an accumulation of lipids and calcium in the media independent of the atherosclerosis in the intima. Analyses of intimal lesions in atherosclerotic aortas may be summarized as follows. The lesions were separated as much as possible from the normal medial and intimal tissues and classified as fatty, fibrous and calcified plaques and atheromatous ulcers. The first type was the streaked or nodular yellow deposit, in which the lipid material was mainly intracellular and the intima was



slightly thickened but not appreciably scarred. The second type had raised nodules or diffuse plaques covered with tough fibrous material with the typical pearly luster, necrosis and pooling of lipids. The third type consisted of brittle calcified plaques with centers of soft necrotic material, characteristic of these late lesions. The atheromatous ulcers were ruptured or at the stage of incipient rupture, and contained, in addition to the soft necrotic lipid material, clotted blood and some visible calcification. These types probably represent successive stages of the disease.

TABLE 4—Values of Constituents of Intimal Lesions According to Age

Type of Lesion	No	Age	Mois- ture*	Lipid Ex- tract†	Composition of Lipid Extract							Nonlipid Residue		
					Cholesterol‡			Phosphatide‡			Galac- toside‡	Ash‡	Ash†	Cal- cium†
					Free	Ester	Ether Sol- uble	Ether Insol- uble	Total					
Fatty plaques														
	2	22	77.4	25.0	14.9	32.9			30.7	2.76	6.6	2.77	0.13	
	26	35		30.8	11.5	44.4	11.6	4.2	15.8	5.20	0	1.67	0.23	
	9	49	67.8	25.7	19.0	24.1	9.1	11.5	20.6	6.91	3.7	8.87	2.22	
	27	67	57.2	22.2	17.0	47.2			17.8	7.00	4.2			
Fibrous plaques														
	7	43	64.9	9.55	13.0	25.9	7.2	13.1	20.3	3.73	2.91	19.5	7.15	
	10	49	67.3	37.8	19.9	65.2	1.05	11.6	13.1	5.95	3.8	12.8	4.63	
	19	67	66.7	37.4	16.4	51.9	3.53	10.0	13.5	3.44		8.28	2.56	
	20	68	62.8	30.5	21.6	50.5	10.9	0	10.9	3.72	2.90	4.53	1.43	
	28	78	70.9	20.6	16.0	35.5			12.8	4.50	5.2			
Calcified tissues														
	15	58	48.7	14.4	18.1	50.8	1.54	14.4	15.9	3.7	3.1		18.9	
	27	67	28.6	9.23	20.0	51.0			13.7	5.7	2.3	65.2	37.1	
	20	68	45.2	14.2	20.1	54.9	5.91	0	5.91	3.92	4.52	45.9	17.7	
	22	72	27.5	10.3	18.1	43.1	5.98	3.5	9.5	3.04		66.8	24.9	
	24	78	34.1	13.8	20.6	44.1	0.88	13.1	14.0	6.40	3.96	65.0	25.0	
	25	84	47.5	14.6	30.0	29.2	4.10	14.2	18.3	3.70		62.3	22.1	
Atheromatous ulcers														
	29	51		33.3	23.8	42.8	6.0	10.6	16.6	5.8	0	39.2	13.8	
	30	68		35.9	27.0	46.5	4.2	11.0	15.2	4.0	0.4	24.1	8.3	
	21	71	62.1	32.4	29.8	31.5	3.70	11.7	15.4	2.70	2.91	33.1	12.3	
	23	75	59.4	42.5	23.7	40.6	8.37	5.75	14.1	4.10	5.6	17.2	6.0	

\* The value is given as percentage of wet tissue  
† The value is given as percentage of dry tissue  
‡ The value is given as percentage of total lipid

The results of the analyses are in tables 4 and 5. The values were determined as described for media except that the values for the lipid constituents represent the percentage of the total lipid rather than of the dry tissue. As the disease progresses through fibrous thickening, calcification and atheroma, there is an increase of free cholesterol as well as of cholesterol esters up to a certain level, then a decrease in the latter is noted. This confirms the observations of Meeker and Jobling who noted a decrease in the ratio of cholesterol esters to free cholesterol in the late stages. In these analyses the decrease in the ratio

is not so great. The total phospholipid remained relatively constant in the various lesions, but within this lipid class extremely wide variations were noted between the proportions of ether-soluble and ether-insoluble constituents. Here, as in the media, there was a decrease in the ether-soluble phosphatides and an increase in the sphingomyelin fraction. The high proportion of sphingomyelin in many of these extracts is significant because previous workers have termed this fraction lecithin. Some anomalies are noted with regard to the phospholipid fractions when different types of lesions from the same aortas are compared. For example, three tissues were analyzed from aorta 20: normal media and calcified and fibrous tissues from the intima. Ether-soluble phospholipids were absent, and the phospholipid fractions were low. The extracts from the media and intima of aorta 10 were high in ether-soluble phospholipids, whereas the fibrous plaques contained only a small pro-

TABLE 5—Average Values of Constituents of Intimal Lesions

Type of Lesion	Mois- ture*	Lipid Ex- tract†	Composition of Lipid Extract Corrected for Ash Content							
			Phosphatide‡					Fatty Acids, Neutral		
			Cholesterol‡		Ether Sol- uble	Ether Insol- uble	Total	Galac- to- side‡	Fat, etc.‡	Cal- cium‡
			Free	Ester						
Normal intima	71.6	14.4	14.2	38.6	13.7	6.4	20.1	8.0	19.1	0.23
Early fatty plaques	67.5	25.9	16.2	38.5	10.8	8.2	19.0	5.8	20.5	0.86
Fibrous plaques	66.5	27.2	18.1	47.5	5.9	9.0	14.9	4.5	15.0	3.94
Calcified tissues	38.6	12.8	21.9	47.2	3.9	9.3	13.2	4.6	13.1	24.3
Atheromatous ulcers	60.8	36.0	27.2	42.1	5.8	10.2	16.0	4.3	10.4	10.1

\* The content is given as percentage of wet tissue.

† The content is given as percentage of dry tissue.

‡ The content is given as percentage of total lipid.

portion of this fraction. The galactoside fraction varied widely and inconsistently in the lesions.

There was also a sharp decrease in the "neutral fat-fatty acid" fraction. The values for this fraction were obtained by difference; thus, its actual composition is unknown. It consists probably of free fatty acid, a small proportion of neutral fat, and some unsaponifiable matter related to cholesterol.

There were no great or abrupt changes in the lipid composition with advancing severity of the lesions. Even in extremely necrotic and calcified lesions the difference in lipid composition from normal tissue did not vary greatly from the individual variations found in extracts of lesions of the same type.

As in the normal intima the amount of calcium found in the early deposits is small. Not until fibrous thickening and pooling of lipids occur is there an increase in calcium content. High calcium values are found thereafter, especially where visible calcification has occurred.

## COMMENT

If the lipid deposits in atherosclerosis of the aorta arise through infiltration by the plasma without selective activity of the tissues, the early deposits in which little necrosis has occurred should approximate in composition the lipid portion of the blood plasma. The most comprehensive study of the lipid composition of human plasma is that of Page, Kirk, Lewis, Thompson and Van Slyke<sup>23</sup>. They determined free and total cholesterol and phospholipid in the petroleum ether extracts of blood plasma from normal men of ages from 20 to 101 years. The analyses, made according to the gasometric methods of Van Slyke and co-workers,<sup>24</sup> are the best figures available at present. The cholesterol and phosphatides were determined directly, the "neutral fat" fraction was computed by difference between the total lipids and the phosphatides, cholesterol and cholesterol esters. As cerebrosides were not determined by these authors, this fraction has been included in the neutral fat fraction in our figures to provide a better basis of comparison between the two sets of values. There is a striking agreement between these figures, as shown in table 6, despite the differences in analytic procedures.

TABLE 6—*Comparison of Lipid Composition of Blood Plasma and Arterial Tissue*

	Blood Plasma*	Intima*	Early Plaques*	Media*
Free cholesterol	14.1	14.2	16.2	17.3
Cholesterol esters	38.3	38.6	38.5	16.7
Phospholipids	22.8	20.1	19.0	34.1
Neutral fat, etc	23.3	27.1	26.3	31.9

\* The values are given as percentages of total lipid

In a recent article Kirk<sup>17</sup> reported on the distribution of the three individual phospholipids and cerebrosides in blood plasma. Among these four constituents are the same large variations we found in the tissue lipids, thus affording additional, though indirect, evidence that the latter are derived directly from the plasma.

The close agreement in composition between lipid extracts from the plasma and the normal intima and the wide differences between the lipid extracts from the latter and the media indicate that the lipids of the intima originate in the plasma rather than in the protoplasm of the cells. The main portion of the lipids of the intima, in other words, is extracellular. The foregoing comparison supports the validity of Anitschkow's hypothesis that the intima is freely permeable to the lipids.

<sup>23</sup> Page, I. H., Kirk, E., Lewis, W. H., Thompson, W. R., and Van Slyke, D. D. *J. Biol. Chem.* **111**: 613, 1935.

<sup>24</sup> Kirk, E., Page, I. H., and Van Slyke, D. D. *J. Biol. Chem.* **106**: 203, 1934.

of the plasma and that the intima wall exerts no selective action on these lipids. The close agreement between the composition of the extracts from the normal intima and that of the early fatty deposits suggests further that these deposits arise through nonspecific deposition of the plasma lipids. The changes in lipid composition which occur with advancing severity of the disease may be the result of several simultaneous and different processes. These are phagocytosis, with other attempts of the tissues to remove a foreign substance, and necrosis resulting from altered nutrition or from chemical action of the lipid substance deposited<sup>25</sup> in the tissues. These processes are secondary to an initial deposition of lipid, as is borne out by the appearance of stainable lipid in the intima as the earliest change observed microscopically.

Cholesterol, the only specific lipid detected microscopically, has been stressed as the important substance in atherosclerosis. The dramatic consequences of cholesterol feeding in rabbits have emphasized further its importance. The analytic results reported here indicate, however, that not merely cholesterol but lipid substances in general are involved in atherosclerosis. They indicate that the effect of cholesterol in the production of experimental atherosclerosis is not direct but is exerted through its effect on the physicochemical state of the lipids as a whole in the plasma. This role of cholesterol in atherosclerosis may be verified by experiments in rabbits and by analyses of aortic lesions similar to those reported here. Although there is reasonable evidence that the lipids in the lesions of atherosclerosis originate in the blood plasma, the cause of their deposition remains obscure.

#### SUMMARY

The lipid and calcium contents of the media of the human aorta increase with age. The increase is not correlated with the degree of atherosclerosis of the intima.

Intima without lesions has larger amounts of lipid and smaller amounts of calcium than the corresponding media.

With increasing severity of the atherosclerotic lesions of the intima the proportions of free and combined cholesterol increase until the onset of necrosis, then the proportion of the combined cholesterol decreases. Also, there are increased proportions of ether-insoluble phospholipids and calcium and decreased proportions of ether-soluble phosphatides, galactosides and fatty acids.

The proportions of the individual lipid constituents in the intima and in the simple fatty deposits of the intima correspond closely with those reported for these substances in blood plasma. These relations imply that the lipid deposits in the intima are the result of nonselective infiltration and precipitation of lipids from the plasma of the blood.

# MORPHOGENESIS OF EXTRASKELETAL OSTEOGENIC SARCOMA AND PSEUDO-OSTEOSARCOMA

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The occasional occurrence of so-called extraskeletal osteogenic sarcoma is well known. The tendency of forms of traumatic myositis ossificans to simulate malignant osteogenic sarcoma and rarely to become such is also recognized. Extraskeletal osteogenic sarcoma not related to traumatic myositis and osteosarcoma probably originating in heterotopic bone deposits in various viscera are also familiar, and these plus a number of tumors resembling osteogenic sarcoma and readily confused with that disease are of sufficient interest to warrant an attempt at analysis of conditions under which they arise and peculiarities leading to their curious morphologic appearance.

Early medical observers recognized the rarity of osseous tumors unrelated to the bony skeleton. In 1700 Boneti<sup>1</sup> described an osteoma of the mammary gland. Morgagni,<sup>2</sup> in 1769, Astley Cooper,<sup>3</sup> in 1845, and Johannes Muller,<sup>4</sup> in 1838, described ossified tumors of the female breast. Numerous reports concerning bone and cartilaginous tumors of the mammary gland appeared during the latter part of the nineteenth century. Raso,<sup>5</sup> in 1937, was able to collect 75 cases of "chondro-osteoma" and "chondro-osteosarcoma" of the human mammary gland. He cited 8 cases of so-called osteogenic sarcoma of the breast which he found recorded in the literature.

Pick,<sup>6</sup> Funkenstein,<sup>7</sup> Solario<sup>8</sup> and Biorders and Pemberton<sup>9</sup> have reported cases of osteogenic sarcoma primary in the thyroid gland.

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From the Memorial Hospital

1 Boneti, T. De ventris tumore, in *Sepulchretum, sive anatomia practica ex cadaveribus morbo denatis*, Geneva, Cramer et Perachon, 1700, vol 3, sect 21, obs 61, p 522.

2 Morgagni, J B. The Seats and Causes of Diseases, translated by B Alexander, London, A Miller and T Cadell, 1769, vol 3, letter L, obs 41, p 63.

3 Cooper, A. The Anatomy and Diseases of the Breast, Philadelphia, Lea & Blanchard, 1845, p 47.

4 Muller, J. Ueber den feinem Bau der krankhaften Geschwulste, Berlin G Reimer, 1838, p 48.

5 Raso, M. *Pathologica* **29** 229, 1937.

6 Pick, F. *Ztschr f Heilk* **13** 71, 1892.

(Footnotes continued on next page)

Coley,<sup>10</sup> Rhoads and Blumgart,<sup>11</sup> Mallory<sup>12</sup> and others have discussed osteogenic tumors occurring in the soft parts, many of them histologically identical with osteogenic sarcoma of the bony skeleton. Most of these tumors were described as clinically benign, although often the patients were inadequately followed. Coley pointed out that a few of the osteogenic tumors which arise on the basis of traumatic myositis are clinically malignant, and the patients have pulmonary metastases. Butler and Woolley<sup>13</sup> reported a metastasizing osteogenic sarcoma which developed in a bulky calcified hematoma. Ewing has often mentioned osteogenic sarcoma developing in a calcific deposit of old phthisis bulbi. Heterotopic bone formation is evidently common in phthisis bulbi.

The quoted literature and other papers which for brevity's sake are not cited here offer little or nothing concerning the mechanism of the morphogenesis of these curious tumors. Thus osseous and cartilaginous tumors of the uterus, ovary, lung, kidney, pleura, meninges and other tissues are described, but their morphologic nature is unexplained.

Experimental workers in the early part of the century presented histologic evidence of metaplasia of connective tissue into bone. More recent investigations, such as those of Asami and Dock,<sup>14</sup> Huggins<sup>15</sup> and others, have stressed the concept of metaplasia of connective tissue into bone under some unknown influence. Leriche and Policard<sup>16</sup> recalled the old observations of von Recklinghausen on the relation of calcification to slowing of the lymphatic flow. They pointed out that in ossification following fracture the most important morphologic indication in the region to be ossified is the appearance of an edematous state, hard and lardaceous, and peculiar to connective tissue about to ossify. They noted that a gelatinous substance infiltrates the interstices of the connective tissues. They emphasized the fact that zones which may be expected to ossify soon have few vessels and they linked the aforementioned edema to defective circulation and stagnation of the connective tissue fluids. Newly formed spicules of bone are said to be situated as far as possible

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7 Funkenstein, O. *Virchows Arch f path Anat* **171** 34, 1903

8 Solaro, G. *Clin chir* **21** 1101, 1913

9 Broders, A. C., and Pemberton, J. de J. *Surg, Gynec & Obst* **58** 100, 1934

10 Coley, W. B. *Ann Surg* **57** 305, 1913

11 Rhoads, C. P., and Blumgart, H. *Am J Path* **4** 363, 1928

12 Mallory, T. B. *Am J Path* **9** 765, 1933

13 Butler, F. E., and Woolley, I. M. *Radiology* **26** 236, 1936

14 Asami, G., and Dock, W. *J Exper Med* **32** 745, 1920

15 Huggins, C. B. *Arch Surg* **22** 377, 1931

16 Leriche, R., and Policard, A. *The Normal and Pathological Physiology of Bone Its Problems*, translated by S. Moore and J. A. Kev, St. Louis, C. V. Mosby Company, 1928

from the blood capillaries which enter the nonossified connective tissue spaces. These authors likewise noted that an increase in circulation of the blood results in resorption of bone, that new bone formation may follow concomitant rarefaction of either bone or calcified elements and that rarefaction either of bone or of calcified substance may lead to new bone formation in the neighboring connective tissue. The relation of blood supply to normal calcification of bone has lately been emphasized by Blair<sup>17</sup>

We are not interested in merely adding to the number of reported cases of ossified soft part tumors but rather in determining the mechanism of their morphogenesis. Study of a number of extraosseous tumors which bear at least some resemblance in certain of their areas to osteogenic sarcoma convinces us that these areas present several modes of origin.

The simplest origin is seen in the ossified basal cell carcinoma. In the examples of this tumor in our possession a metaplastic origin of the bone in the sense of true metaplastic epithelial bone cannot be seriously considered. The earliest indication of impending ossification is found in a thick hyaline deposit about capillaries and tiny arterioles. The hyaline substance is not at this stage confined to the pericapillary area but is found in the capsular connective tissue of the tumor and in the walls of major vessels of the vicinity. Whether changes appear first in the larger or in the smaller vessels cannot be determined from this material. The hyalinization about capillaries seems to lead to total obliteration, and in many hyaline strands no lumen can be identified. The process may involve a considerable area. There follows a peculiar bluish edematous degeneration of the hyaline cord, and calcific granules are deposited in this matrix. The calcific plaque is not confined to the hyaline tissue of the tumor stroma but may be found in the walls of surrounding larger vessels. Where readily identifiable vessels remain they show a definite tendency to assume a thin-walled cavernous form, possibly a dilatation following more distal obstruction through hyaline obliteration. Be that as it may, it is clear that one of the conditions for ossification and calcification in this tumor is circulatory failure caused by (1) hyaline obliteration of capillaries and (2) development of cavernous vessels favoring stasis and consequent probable anoxemia. A feature which escapes explanation is the cellularity of the residual periosseous stroma, which may approach that of a true neoplastic process and in appearance may be linked, perhaps, to active myositis ossificans. Osteoblast condensation may be noted. Numerous multinucleate giant cells testify to resorptive activity.

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17 Blair, H. C. Surg., Gynec. & Obst. 67: 413, 1938.

It is the cellularity of the interosseous stroma which causes difficulty in deciding whether the bone is formed in connective tissue or whether it is metaplastic bone in the sense that the epithelial cells of the tumor become bone cells. The separation of tumor cells and interosseous stroma is difficult. Tumor cells invade the stroma and break up into fine strands and isolated elements which when molded by surrounding spindle-shaped cells tend to assume a spindle form, and one derives the false impression that these cells are entering into bone. Ulceration, granulation tissue and infection further confuse the picture. Bone development, however, is clearly defined wherever it is seen to proceed independent of epithelium.

Such a lesion, despite its superficial resemblance to a bone-forming tumor, is of course nothing of the sort. The bone is not neoplastic bone in the usual sense. It is quite possible, however, that this sort of bone formation passes over into neoplastic osteogenesis. Indeed, we have good evidence that essentially similar mechanisms obtain in tumors which cannot be distinguished on mere morphologic grounds from osteogenic sarcoma.

An interesting combined cancer and osteogenic sarcoma was recently reported by Budd and Breslin<sup>18</sup>. They interpreted it as a mixed tumor because the osteogenic tissue exhibited as much evidence of invasion and rapid and autonomous growth as the carcinoma. The difficulties of interpretation of the material are evidenced by the fact that Ewing, Mallory and Masson, all of whom saw the material, reached different conclusions as to the nature of the process. Since the sections sent by Budd to Ewing are still in the Memorial Hospital collection, they have been restudied by us in connection with sections from other tumors of pertinent types, and our views coincide with those of Ewing, although his letter is not sufficiently specific to indicate his own detailed explanation of the histologic picture. The description of Budd and Breslin indicates that the tumor was one of long duration in a state of essential quiescence (twenty-eight years). It is therefore most unlikely that it was mammary cancer, for if it were, we should have to assume that mammary cancer developing in a young woman of 30 years—a period of unusual malignancy for the average mammary cancer—grew slowly for twenty-eight years. Masson and Ewing classed the tumor as mammary. Mallory believed it to be mammary, since he thought it originated in a fibroadenoma, yet Budd and Breslin did not describe it as in the breast but rather as situated in the wall of the chest 3.5 cm. below the midclavicular area. It was *attached to* and elevated the skin.

Budd's sections are interesting. In one there is a small focus, well outside the main tumor, where a small sweat gland appears with a dense

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18 Budd J. W., and Breslin, F. J. *Am J Cancer* **31** 207, 1937



hyaline stroma This minute area resembles a cylindriomatous sweat gland tumor though it is scarcely large enough to be a tumor at all Nevertheless this finding gives a hint as to the nature of the main tumor and the basis for its general resemblance to mammary cancer It also affords a reason, based on the known behavior of sweat gland tumors, why the tumor grew so slowly There is evidence that the main tumor had long possessed a dense hyaline connective tissue capsule and that extensive hyaline alterations in the stroma had been present Also the surrounding vessels are markedly thickened In one section there is evidence of widely dilated lymph (?) spaces with some blood, or possibly of blood spaces, suggesting the stasis seen in other lesions where ossification or calcification has supervened The question in our minds is whether the bone is being formed or being destroyed, and it appears to us that destruction is obvious, whereas new formation of bone is at least doubtful We are not convinced that osteoblasts are present, and, on the other hand, clasmotocytes are numerous The latter are found also in the midst of areas of obvious cancer, and the only known reason for their being present in such areas is that these zones previously contained bone or calcified material which had disappeared under the cancerous invasion

Differences of interpretation are possible as to the relation of the epithelial tumor cells to the bone It is certain that in cross section many tumor cells appear to be surrounded by hyaline matrix or bone, but, on the other hand, it is also clear that malignant tumor cells are to be found invading bone from without, in penetrating strands, and that cells apparently surrounded by bone may be found in rows of two or three like an epithelial tumor Where bone is farthest removed from surrounding cancer, we do not find obvious cancer cells in the bone but only bone cells or empty spaces In our opinion, the malignant cells found within the bone are cells of an epithelial tumor, the spindle cells represent metaplastic epithelial cells, and we therefore agree with Ewing that the tumor is purely epithelial We believe that the resemblance to osteogenic sarcoma is striking but that on more minute analysis it falls down and that we are dealing with an old calcified, ossified epithelioma, probably of sweat gland origin, in which cancer has developed, sweat gland cancer often having a close resemblance to classic mammary cancer for obvious reasons, and that the resemblance to osteogenic sarcoma is the result of rapid invasion and dissolution of preformed stromal bone by cancer Thus the process differs from what is usually considered a malignant mixed tumor We apologize for offering an unsolicited fourth opinion on this interesting tumor After we had reached our opinion, there appeared in the literature a description of a similar tumor by Tudhope,<sup>19</sup> concerning which he reached a conclusion nearly similar

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19 Tudhope, G R J Path & Bact 48 499, 1939

to ours, the difference being that his tumor was originally definitely mammary fibroadenoma

A resemblance to osteogenic sarcoma is seen in a curious, exceedingly vascular mammary cancer in which the events leading to the histologic similarity between the carcinoma and osteogenic sarcoma are at least partially traceable

The tissue surrounding the tumor is made up of hypertrophic mammary lobules with their branches widely separated by edematous connective tissue infiltrated with lymphocytes. The mammary fat tissue is unusually vascular and almost lipomatoid. In certain of the lobules small invaginations of duct epithelium have developed. Both in these invaginations and in the lumens of the glands are prominent calcific psammoma bodies. The indications are that the deposition of calcium occurs in areas of intraductal hemorrhage. A source of calcium for subsequent events is thus present.

The tumor itself is obviously a low grade comedo carcinoma where it can be recognized as carcinoma at all. Now evidently a circulatory accident has occurred which has resulted in communication between the ducts, lined by comedo carcinoma, and vessels, with conversion of part of the tumor into a markedly telangiectatic structure. It is possible that in the telangiectatic area a calcium surcharge is present. Indications of an excess of calcium have already been noted. At any rate, large numbers of epulis-like giant cells are present in the area, the resemblance of the lining cells to comedo carcinoma is so altered that the separated cells cannot readily be identified as epithelial, an effusion of blood together with cells occurs into the loose connective tissue, where resemblance to epithelium is further lost. Tumor giant cells, epulis-like giant cells, telangiectasia and loose tumor cells, no longer recognizable as epithelial in origin, complete the resemblance to telangiectatic osteogenic sarcoma.

In another tumor of similar nature the epulis giant cells are so numerous that wide areas duplicate the structure of benign giant cell tumor of bone.

In our cartilage-containing tumors of the human breast, tumors resembling the common cancer of the canine breast, we find no satisfactory explanation for the appearance of the cartilage. It seems to be associated with a mucoid edema of the spindle cell portion of a mixed "carcinosarcoma," obviously in some instances a development in a pre-existing fibroadenoma. The edematous connective tissue is rather vascular, being well equipped with capillaries having only an endothelial wall. The evidence of stasis previously described is not found in these edematous areas, and occlusive hyalinization of vessels is not seen. The static type of circulation may appear later, after the formation of rather adult-looking cartilage, but then it forms a sort of cavernous plexus about

the cartilaginous areas. The evidence in our material is insufficient to indicate that the circulatory changes are causal, and the reason for the characteristic cartilaginous development in these tumors is totally obscure. To call them mixed tumors or to invoke a teratoid origin evades the question.

It is evident from another mammary tumor—a pure epithelial “carcinosarcoma”—that the static type of circulation with hemorrhage is not alone sufficient to produce calcification or ossification in the mammary tumor. The lesion is a circumscribed solid adenocarcinoma made up of plexiform sheets of closely packed small polyhedral cells. In places the closely packed areas of epithelial cells give way to progressively looser areas where the picture changes from one in which the resemblance to condensing mesenchyme is striking to one in which the epithelial character of the cells is lost through their becoming mingled diffusely with mononuclear phagocytes, plasma cells, loose elements of areolar tissue, giant cells and blood. In these loose areas there is hemorrhage, the vessels are widely dilated and have only a single endothelial layer and one gets the impression of marked stasis. However, no hyaline tissue is found, nor does one find evidence of calcium. Apparently, despite the presence of a circulatory picture found in other instances in which ossification has occurred, the connective tissue fails to provide through preliminary hyalinization an ossifiable medium.

The relation of heterotopic bone formation to blood vessels is seen in the evolution of a curious tumor<sup>20</sup> of the lower extremity. A series of biopsy specimens show the progress of the lesion. The process begins in multiple small, lobule-like capillary angiomas (fig 1). These lie in fat tissue, but the fat lobules appear rather fibrosed, and in the interstices between the capillaries the active cells appear to be spindle cells of the connective tissue rather than fat cells. In each of the smallest lobules is a central vessel of the caliber of a small arteriole. This branches in all directions toward the periphery of the circumscribed lobule, the terminal branches being capillary in type. The larger vessels show a poorly developed muscularis, the cells of which are poorly differentiated from fusiform or branched connective tissue cells occupying the intervening loose areolar tissue, in cross section the cells of the muscularis of certain of the vessels suggest the myoid cells of the glomus. The intervening areolar tissue is somewhat edematous and contains a fine fibrillar deposit. Not all of the vascular lobules show the capillary structure. Some seem entirely made up of thick wall arterioles, and the lesion resembles more a varix.

As in previous examples of bone formation, the first event seems to be extensive progressive hyalinization of the capillary wall. This proceeds until the layer of hyaline material exceeds the diameter of the vessel in

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20 Dr. Howard Permar gave us permission to use this case.

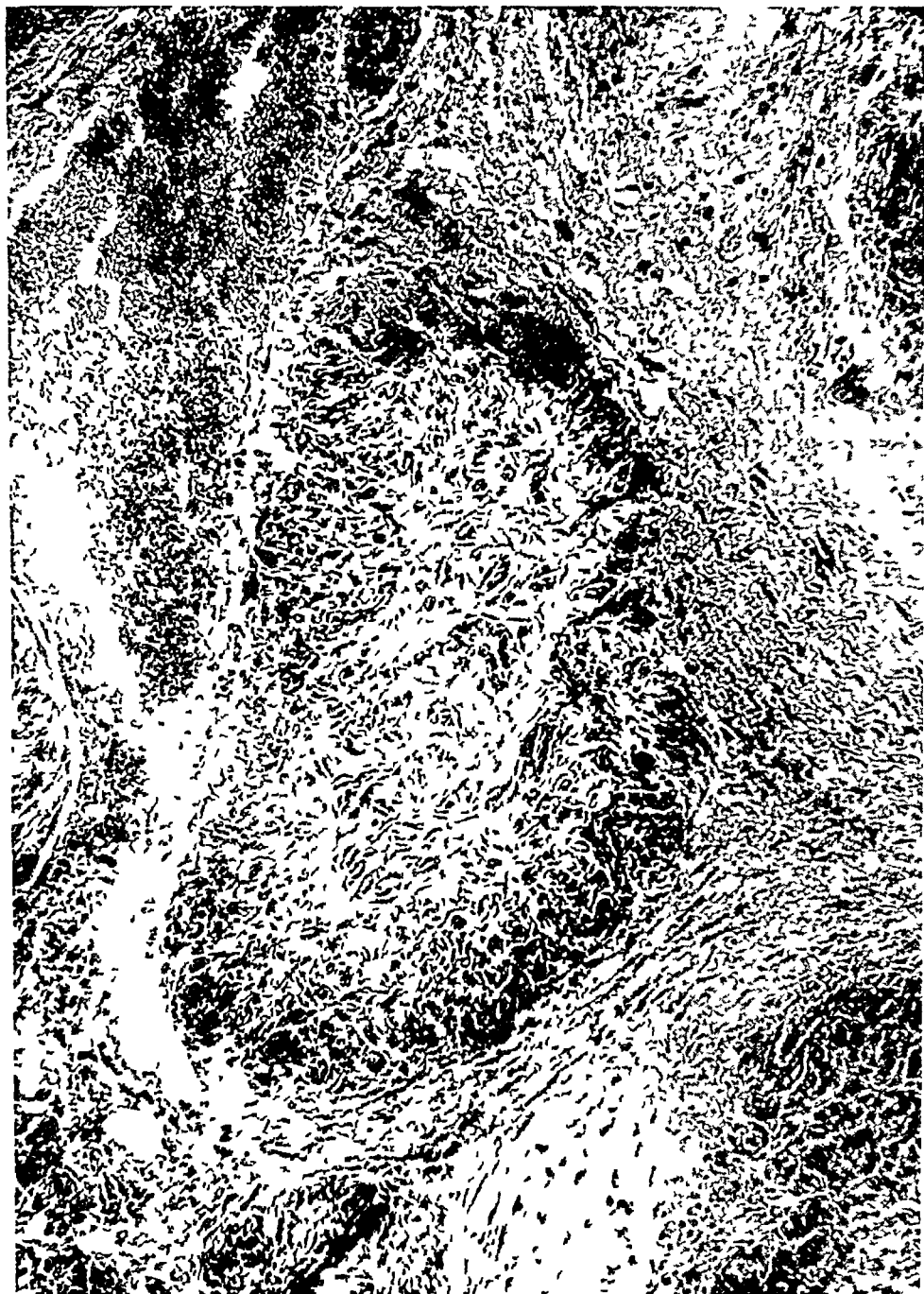


Fig 1—One of a group of lobular angiomas, prior to the development of perivascular hyalinization

thickness or even until obliteration of the capillary has occurred (fig 2). The entire capillary bed may disappear, and the residual picture may be that of a cavernous angioma surrounded by thick hyaline material. It is in these hyaline areas that calcification and poor ossification occur.

The neoplastic process in its malignant phase (fig 3) develops in the individual hyalinized or calcified or ossified vascular lobules as a malignant multicentric tumor. We cannot be certain of the exact cells of origin, i. e., whether muscle or connective tissue, but they are clearly not cells of the vascular endothelium. We believe them to be connective tissue cells. The structure of the tumor depends largely on the character

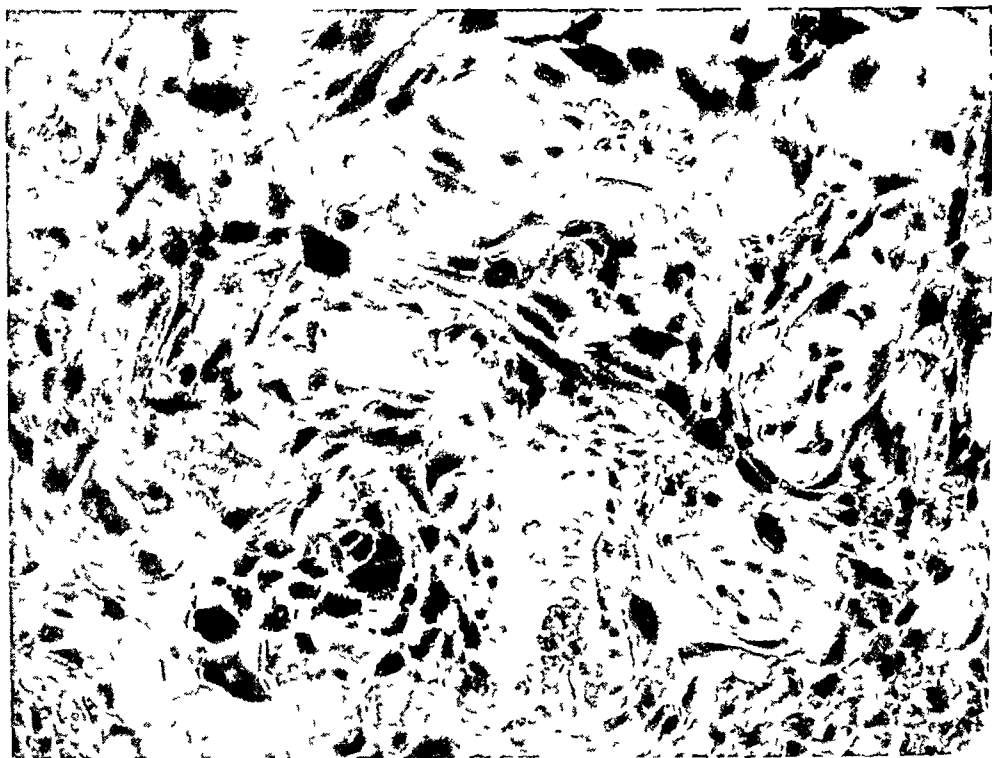


Fig 2—Later stage showing perivascular hyalinization and partial occlusion of vessels. Perivascular tumor cells are present.

of the process in the vascular lobule at the time the malignant phase begins. In the presence of extreme hyalinization the appearance suggests that of an osteoid osteosarcoma. With marked calcification or ossification the resemblance is to osteogenic sarcoma (fig 4), although nowhere is there clearcut evidence that malignant tumor cells are forming bone, they rather occupy interstices between hyaline bundles and calcified matrix, which they invade and in which they develop. The resemblance to bone sarcoma is intensified by the presence of numerous tumor giant cells together with giant cells of the epulis type both in the hyaline and in the calcified areas.

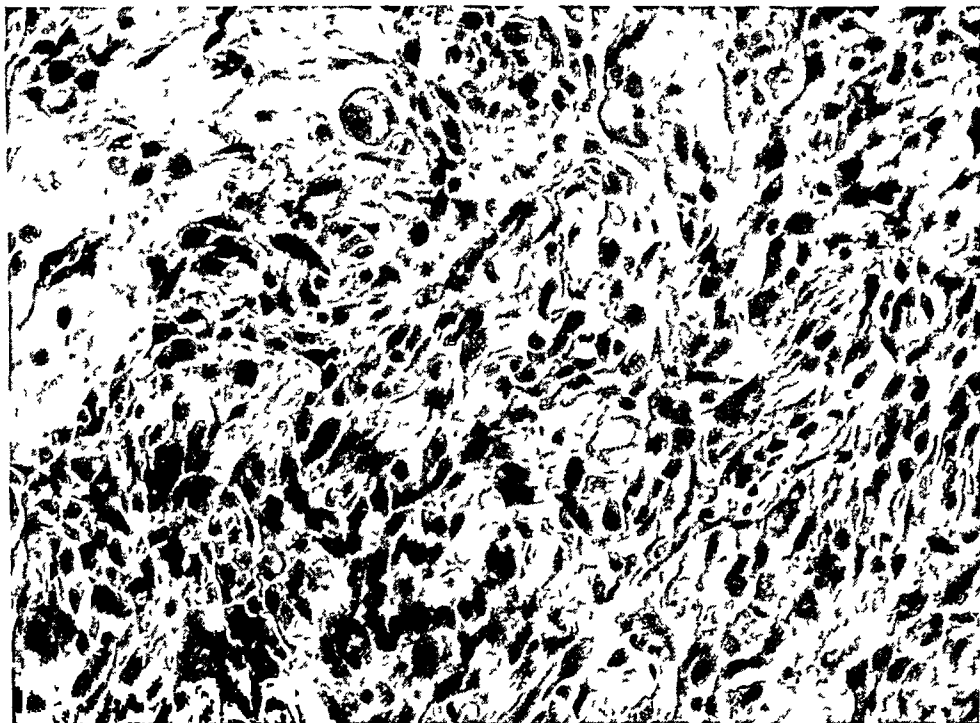


Fig 3—The same tumor, more atypical and malignant looking, and showing resemblances to osteoid sarcoma

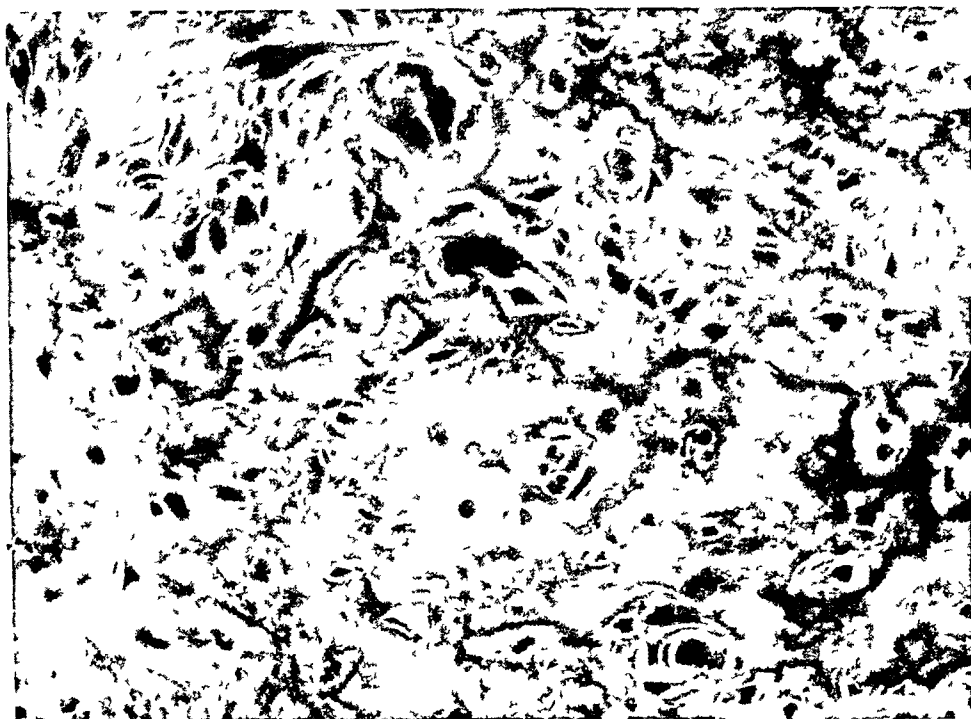


Fig 4—One of many areas of bone formation, where the lesion resembles osteogenic sarcoma

Here the malignant tumor is something which has been added to an originally benign angioma which underwent degenerative changes so altering the circulation that calcification and ossification occurred. The malignant tumor developed in multiple lobules under an unknown stimulus. Our study has clarified conditions responsible for the histologic character of the malignant tumor but not its etiologic nature. No source of calcium surcharge is known, the tumor was in close proximity to the tibia, which may have been injured in early operations.

This relation of vascularity to calcification is seen in another interesting tumor in the Memorial Hospital collection. The tumor is a uterine angiomatoma whose original structure probably corresponded to that

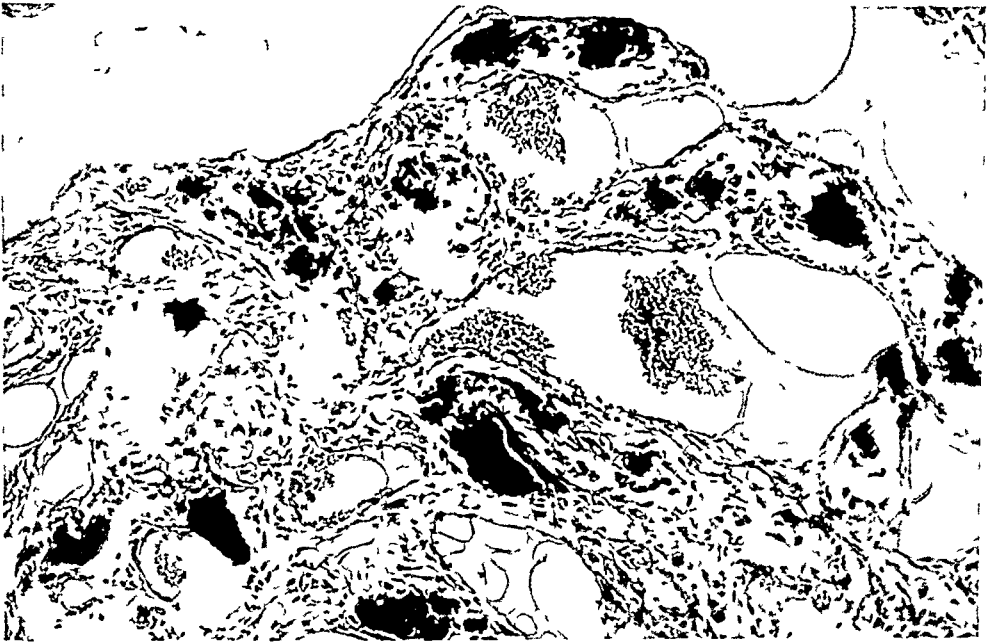


Fig 5—Areas of telangiectatic cavernous vessels found between myomatous bundles, calcification, now showing signs of resorption, in the interstices, cells resembling bone cells in the calcific material

described by Pusch<sup>21</sup> as "ramifying angiomatoma of the uterus." In this tumor the myomatous elements are arranged about cavernous blood vessels which in some areas constitute essentially a cavernous angioma surrounded by a connective tissue sheath inside and a myomatous sheath outside. The major vessels outside the tumor are greatly thickened. Bone is not laid down where the vessels are thick walled, sclerotic and hyalinized, but rather it seems to appear where they are thin walled and aneurysmal and where the picture suggests stasis (fig 5). There is no evidence that the muscle cell is concerned. The calcification occurs in the connective tissue, and the connective tissue cell becomes

<sup>21</sup> Pusch, L. C. *Am J Obst & Gynec* 24:907, 1932

incorporated as a bone cell. No cells resembling osteoblasts are seen. In some areas, after bone formation has occurred there is evidence of vascular occlusion with infarction, death of bone and reaccumulation of lime salts in the hyalinized connective tissue.

Although the benign tumor was clearly a perivascular uterine myoma, the malignant tumor which developed in the areas of calcification and ossification appears to be a connective tissue tumor. We first accepted the specimen as one of osseous metaplasia of myomatous tissue or osseous metaplasia in a myosarcoma but after much study were forced to abandon that concept. The malignant change begins in the connective tissue in multiple areas. It can be followed in separate perivascular

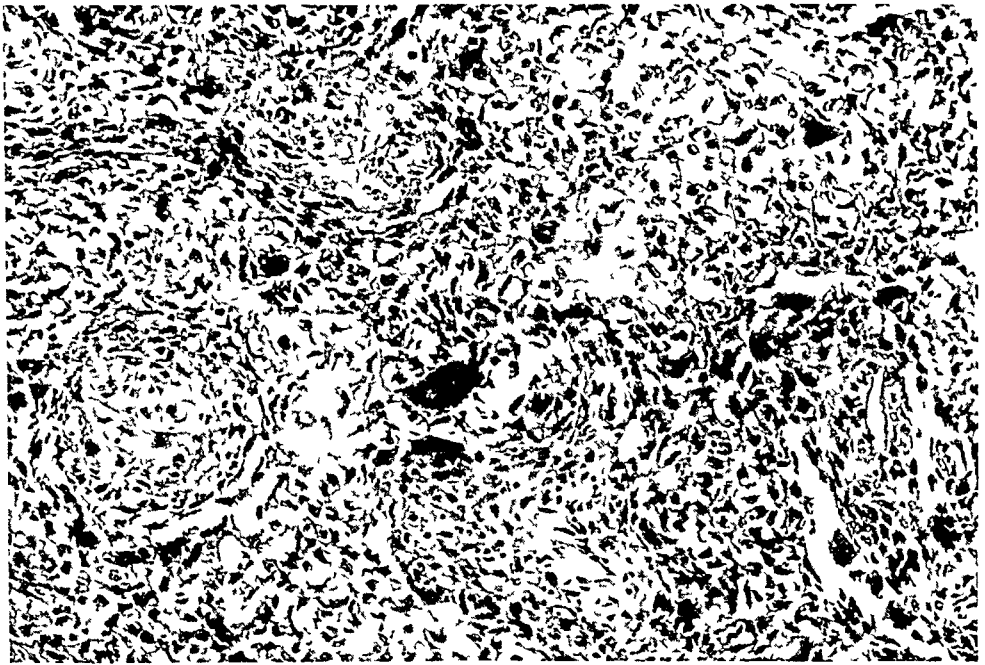


Fig. 6—Actively malignant area of the same tumor showing a structure indistinguishable from osteogenic sarcoma.

units surrounded by muscle, which later it invades. Thus the tumor is a connective tissue sarcoma invading preformed membrane bone and calcified and noncalcified hyaline tissue and its resemblance to osteosarcoma is a consequence of this fact (fig. 6). As usual the similarity is intensified by the mingling of epulis-like giant cells with true tumor giant cells and the occurrence of masses of hyaline tissue resembling osteoid hyalin, which material, however, we interpret as originally the result of degenerative perivascular hyalinization rather than as a new product formed under the influence of the malignant tumor cell. In fact, the appearance of the tumor cells suggests cells too active in the direction of growth to be concerned in the laying down of collagen. The reason



for the development of a malignant connective tissue, pseudo-osteogenic sarcoma in an angiomyomatous uterus is unknown. The conditions believed responsible for the osteogenesis are traced in part, but these naturally afford no basis for understanding the development of the sarcoma itself.

Barringer and Woodard<sup>22</sup> recently described a peculiar prostatic tumor in the Memorial Hospital series. The lesion presented the combined features of typical prostatic cancer and a calcifying chondromatous tumor surrounded by what at first appeared to be spindle cell sarcoma. Naturally the latter bore certain resemblances to periosteal sarcoma. The blood serum of this patient yielded three to four times the normal level of alkaline phosphatase, although the patient showed neither clinical nor roentgenographic evidence of bone metastases from prostatic cancer. The acid phosphatase showed no increase, and it was therefore suggested that the picture in the prostatic tumor—the calcifying chondromatous portion—was due to some alteration in the prostatic phosphatase to a type capable of inducing calcification. However, we do not know of the relation of phosphatase to the development of cartilage, and although an altered phosphatase may be invoked to explain calcification, it scarcely explains the preliminary development of cartilage.

The sections are interesting. The cancer itself is not unusual. It is a rather malignant-looking adenocarcinoma, solid and alveolar. The cells contain rather more lipid material than do those of the average prostatic cancer. In the midst of the obvious carcinoma is a large mass of amorphous calcific material and about the latter is a zone of fairly normal-appearing cartilage. The cartilage itself is in turn surrounded by a thick zone of dense fibrous tissue, in places almost acellular, in others containing shrunken nuclei like those of quiescent connective tissue cells, and in still others, large malignant tumor cells. The temptation is to regard the lesion as a metaplastic osteogenic sarcoma, and this indeed was our first interpretation.

However, the calcification is occurring in areas where there are no cells which are possibly interpreted as of epithelial origin. The cartilage seems to develop following the appearance of a mucoid edematous state in the dense connective tissue, although we have not been able to assure ourselves that the transition between connective tissue cells and cartilage cells is a clear one. The cartilage cells do not appear definitely neoplastic, although in some areas one might be justified in calling them chondiomatous. Confusion is the result of invasion of preformed cartilage by epithelial tumor cells. The latter occur as single elements and as narrow strands of three or four cells. They show the cohesion of

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22 Barringer, B. S., and Woodard, H. Q. *Tr. Am. A. Genito-Urin. Surgeons* 31: 363, 1938.

epithelial cells and the acidophilic cytoplasm of those of the prostatic carcinoma. Hence the appearance of metaplastic osteogenic sarcoma is the result of confusion of two distinct processes. We are unable to offer any explanation for cartilage in the prostate. The alkaline phosphatase is probably referable to the local calcifying lesion, since no sign of metastases in bones have as yet appeared, fifteen months after operation.

In another prostatic carcinoma conditions which might well have led eventually to the appearance of pseudo-osteogenic sarcoma are apparent. The principal lesion is typical prostatic cancer. However, in numerous areas there are peculiar osteoid changes in the stroma and in the surrounding bladder muscle and connective tissues. These consist in part of hyalinization of muscle and fibrous tissue and in part in extreme perivascular, mainly pericapillary hyaline deposits. In places capillaries are totally obliterated, and the residual endothelial cells are so reduced through atrophy and disappearance that they resemble isolated future bone cells of osteoid trabeculae. Calcification is beginning in certain of these osteoid trabeculae, and in many areas one finds nothing but clusters of cancer cells occupying the interstices of the latter. From a picture of this sort it is but a short step to an appearance which could be readily confused with metaplastic osteogenesis of epithelial origin. No determinations of phosphatase in this tumor are available.

True heterotopic osteogenic sarcoma is found in a large malignant, metastasizing tumor apparently primary in the pericardium at the base.

The patient was a youth of 19 years. His initial symptom was described as an attack of pleurisy six months prior to admission. He stated that he had remained well thereafter until four days prior to admission, when he had a series of hemoptyses followed by cough, dyspnea and swelling of the face, neck, right arm and right leg. Clinical and roentgenographic examinations revealed what was thought to be a bulky mediastinal tumor. Metastases were demonstrable in the soft tissues of the left thigh, and a neurologic consultation furnished evidence of cerebral metastases. Death occurred two months later.

Autopsy revealed what was interpreted as a primary osteogenic sarcoma of the pericardium with metastases to the brain, pancreas, kidney, lungs and soft tissues of the left iliac fossa and left thigh. Pulmonary edema, pleurisy with effusion, thrombosis of the left common and internal jugular veins and massive hemopericardium were terminal events.

In our opinion this tumor is not a teratoid osteogenic sarcoma but one arising as a metaplasia in fibrous tissue. It surrounds the cardiac base and appears to arise from the pericardium. At least the latter can be traced into the tumor as a thin lamina for a short distance, whereupon it becomes lost. Other regional anatomic structures are identified as distinct from the tumor and viscera, the heart, trachea, esophagus and lungs are merely pushed aside. Evidence for preexisting inflam-

mation of thoracic serous membranes was found in the presence of numerous old fibrous pleural adhesions, which required separation by sharp dissection

The tumor did not begin, in all probability, as an osteogenic sarcoma. The active portion is pure fibrosarcoma. The bone-forming areas suggest parts left behind in the course of active growth. They show the structure of osteoid bone developing in hyalinized connective tissue and occasionally well formed dense bone, whose architecture nevertheless reveals its origin in the osteoid trabeculae. Tumor cells are incorporated as bone cells and even tend toward vacuolation and the formation of what resembles marrow fat. Sections stained specifically for fat are not available. In the osteoid and osseous areas the blood supply consists of thin-walled, widely dilated vessels. In the most actively growing periphery of the tumor no such type of circulation is seen. It is not possible, however, to tell with certainty whether the circulatory type favoring stasis precedes, i. e., whether it is a factor in, the osteogenesis, or follows the latter, but we believe it to be a factor, for in certain of the cellular non-bone-forming areas the telangiectatic features are found developing. When the picrosclerous hyaline material can be definitely localized in reference to the markedly dilated vessel, it is seen to be definitely away from it, i. e., in the least well nourished area and separated from the vessel by spindle cell tumor. Completion of the osteoid change finds the dilated vessel and osteoid tissue in much closer approximation. Mucoid-looking edema of the osteoid tissue precedes true ossification. Osteogenesis is absent in the metastases, which show the structure of pure spindle cell sarcoma. Since we have no reason to assume different cell potencies in the metastases, the assumption that osteogenesis in the primary tumor is the result of specific local conditions rather than of specific cell potencies is probably justified.

#### SUMMARY

A number of tumors of soft tissue having an epithelial origin but bearing some resemblance to osteogenic sarcoma are described, and an effort is made to discover morphologic changes of importance which lead to this resemblance. It may be concluded that the most important alterations leading to the assumption of the structure of osteogenic sarcoma are the laying down of dense hyaline tissue, resulting probably in ischemia, and the development of a cavernous telangiectatic type of circulation favoring stasis and consequent probable anoxemia. These features fail to explain the structure of cartilage-containing mixed tumors, and some other mechanism, as yet undetected, must be invoked.

# BLOOD PRESSURE IN EXPERIMENTAL NEPHRITIS PRODUCED BY INJECTION OF NEPHROTOXIC SERUM

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In 1933 Masugi<sup>1</sup> demonstrated that when rats were given injections of serum from rabbits immunized against rat kidney acute glomerulonephritis resulted. Previously nephritis had been produced repeatedly with anti-kidney serum by Lindemann,<sup>2</sup> Pearce,<sup>3</sup> Woltmann,<sup>4</sup> Wilson and Oliver<sup>5</sup> and others. Masugi, however, in reviving and improving on this almost forgotten method, made a much more thorough study. He was the first to show incontestably the close clinical and pathologic resemblance of this disease to human glomerulonephritis. In a second series of experiments, in 1934, he<sup>6</sup> produced a similar form of nephritis in rabbits by injections of anti-rabbit-kidney duck serum. His work has been amply confirmed by many investigators, including Hempnich,<sup>7</sup> Weiss,<sup>8</sup> Smadel,<sup>9</sup> Arnott, Kellar and Matthews,<sup>10</sup> Ehrich,<sup>11</sup> Koranyi and Hamori,<sup>12</sup> Tsuji<sup>13</sup> and Ogawa and Sato<sup>14</sup>.

With the development, for the first time, of experimental nephritis closely simulating the human disease, many new avenues of investigation were opened. In the course of a study of experimental nephritis produced by nephrotoxic serum I have been interested in observing the changes in blood pressure that accompany the disease in rabbits.

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Masugi<sup>6</sup> determined the systolic blood pressure in his second series of experiments, in which rabbits became nephritic when given injections of anti-rabbit-kidney duck serum. He used the capsule method of von Recklinghausen<sup>15</sup> on the denervated ear. The blood pressure in normal rabbits ranged from 60 to 80 mm of mercury. Hypertension was reported in 5 of 6 animals. Maximum rises of 38 and 17 mm were observed in the 2 rabbits with the most intense nephritis. In 3 animals with less marked nephritis elevations of from 9 to 27 mm occurred. In 1 rabbit the disease was very mild, and no rise of blood pressure was observed. When hypertension occurred, it always preceded the onset of albuminuria. In 1 rabbit the pressure returned to normal before the appearance of the albumin in the urine and did not rise subsequently. The longest duration of hypertension was fifty-seven days.

Arnott, Kellar and Matthews<sup>16</sup> determined systolic blood pressure in nephritic rabbits by the carotid loop method of van Leersum<sup>17</sup>. Certain modifications had been adopted as reported in a previous paper by Arnott and Kellar<sup>18</sup>. Thirty animals were studied. Charts and protocols are shown for 5 representative normal animals given injections of five different nephrotoxic serums. Hypertension was invariably observed. The degree depended more on the particular lot of serum used than on the degree of histologic change in the kidney. In some animals the blood pressure rose within twenty-four hours after the injection of nephrotoxic serum, but in others the elevation did not occur until ten days had elapsed, thus showing an inconstant relation to the appearance of albumin in the urine. Hypertension lasted for from two weeks to "a permanent elevation in pressure". From normal levels of 80 to 100 mm of mercury, rises of from 20 to 40 mm were reported.

In a second series of experiments 7 rabbits were subjected to renal denervation and were later given serum known to be potent. Clinically and histologically, the ensuing nephritis was not significantly different from that observed in the previous experiments, but elevation of blood pressure did not occur. In a third group, of 5 animals, renal denervation terminated already established hypertension. They concluded that their results "strengthen the contention that the hypertension of acute diffuse renal disease depends for its occurrence upon the integrity of the renal nerve supply."

15 von Recklinghausen, H. *Arch f exper Path u Pharmacol* **55** 375, 1906

16 Arnott, W. M., Kellar, R. J., and Matthew, G. D. *Edinburgh M. J.* **44** 205, 1937. Arnott and others<sup>10</sup>

17 van Leersum, E. C. *Arch f d ges Physiol* **142** 377, 1911

18 Arnott, W. M., and Kellar, R. J. *J Path & Bact* **42** 141 1936

A somewhat similar study has been reported by Hamori and Koranyi,<sup>19</sup> who came to opposite conclusions. Blood pressure was determined by use of an ear capsule as described by Grant and Rothschild.<sup>20</sup> In the first experiments they made readings on 3 nephritic animals. In the control period the average pressure ranged from 45 to 60 mm of mercury. There was a rise of from 7 to 10 mm during the incubation period, between the injection of serum and the appearance of albumin in the urine. In 2 rabbits a drop toward normal was followed by a secondary rise of 15 and 40 mm of mercury, respectively, following the onset of the nephritis. In the third animal no secondary rise occurred, although the nephritis was severe.

In a second series of experiments<sup>19a</sup> 4 animals were studied. Two were subjected to bilateral and a third to unilateral renal denervation before nephrotoxic serum was given. The dosage was not uniform. The changes in blood pressure were essentially the same as in those animals in which the renal nerve supply had been left intact. They concluded that hypertension is essentially independent of renal innervation, although the levels attained may be somewhat lower when the renal nerve supply has been destroyed.

Smadel and Farr<sup>21</sup> induced nephritis in rats by the injection of anti-rat-kidney rabbit serum. An ear capsule as described by Moberg<sup>22</sup> was used in determining the blood pressure. Hypertension was found to develop in 2 animals in the eighth month of chronic progressive nephritis. From a normal range of from 50 to 60 mm of mercury, pressures in these animals rose to approximately 100. No elevation of blood pressure was found to occur in the acute phase of the disease.

#### METHODS

*Determination of Blood Pressure*—A capsule similar to that described by Grant and Rothschild<sup>20</sup> was prepared. Condom rubber was used, loosely applied, in place of the gold beater's skin originally recommended.

Satisfactory readings could not be obtained when the central artery of the ear was constricted. In order to obtain constant vasodilatation the rabbit was placed on a warm electric pad covered with several layers of muslin. It was held by a towel which was applied firmly but not tightly. In this position the rabbit was usually quiet and comfortable, and the arteries became fully dilated.

The capsule was then applied to the ear. Elevation of pressure within the capsule repelled the flexible membrane, compressing the central artery of the ear until it could no longer be seen to pulsate. The pressure was then allowed to fall

19 (a) Hamori, A., and Koranyi, A. *Ztschr f klin Med* **133** 722, 1938  
(b) Koranyi and Hamori.<sup>12</sup>

20 Grant, R. T., and Rothschild, P. *J Physiol* **81** 265, 1934.

21 Smadel, J. E., and Farr, L. E. *J Exper Med* **65** 527, 1937.

22 Moberg, W. *Skandinav Arch f d ges Physiol* **69** 218, 1934.

gradually. When a distinctly visible pulsation abruptly reappeared, the pressure measured in millimeters of mercury constituted a reading. An interval of three to five minutes was then allowed to elapse, after which the capsule was reapplied to the ear and another reading obtained. The average of three or more consecutive readings varying less than 3 mm of mercury constituted one measurement. Measurements were made at intervals of twenty-four hours or longer on each rabbit.

The foregoing method of determining blood pressure was adopted for several reasons. In principle it is the same as the method employed by Landis, Montgomery and Sparkman<sup>23</sup> and by Pickering and Prinzmetal<sup>24</sup>. Landis and associates,<sup>23</sup> employing a graphic method, found that an ear capsule gave systolic blood pressure readings that were almost identical with those simultaneously obtained in the opposite ear by the method of Hamilton, Brewer and Brotman,<sup>25</sup> both in the normal rabbit and in those that were made hypertensive by abdominal constriction, asphyxia or an injection of epinephrine. The systolic pressure in the central artery of the ear was found to be 12 to 23 mm of mercury lower than that in the femoral artery. Pickering and Prinzmetal,<sup>24</sup> as well as Landis and associates,<sup>23</sup> found the ear capsule method adequate to detect rises in pressure caused by injection of pressor extracts obtained from the kidney.

Figure 1 shows the response of the systolic blood pressure to intravenous injections of a renal pressor extract, prepared by a method similar to that of Landis and associates, as revealed by the blood pressure method employed in this investigation.

Constant dilatation of the central artery of the ear is essential for consistent measurements of blood pressure. In my hands the requisite dilatation has been obtained best by warming the animal. A quiet, dark environment, recommended by Korányi and Hámosi,<sup>12</sup> when used alone was not found to be adequate. In the few animals in which sympathetic denervation of the ears was performed, as recommended by some,<sup>26</sup> continuous full dilatation of the central artery was not obtained.<sup>27</sup> A water-warmed box<sup>23</sup> for heating the rabbits was discarded for an electric heating pad because the use of the latter was simpler, and the animals less frequently became restless and uncomfortable.

The van Leersum method<sup>17</sup> in my hands did not give consistent results from day to day. Moreover, the method is open to criticism, for

23 Landis, E. M., Montgomery, H., and Sparkman, D. *J. Clin. Investigation* **17**: 189, 1938.

24 Pickering, G. W., and Prinzmetal, M. *Clin. Sc.* **3**: 211, 1938.

25 Hamilton, W. F., Brewer, G., and Brotman, I. *Am. J. Physiol.* **107**: 427, 1934.

26 Masugi,<sup>6</sup> Hamori and Korányi.<sup>19a</sup>

27 Grant, R. T. *Clin. Sc.* **2**: 1, 1935.

it is well known that occlusion of the carotid artery may bring about a rise in systemic blood pressure through the activity of the carotid sinus mechanism<sup>28</sup>

*Production of Nephritis in the Rabbits*—Nephrotoxic duck serum was prepared by a modification of the method of Masugi<sup>6</sup> Ducks were immunized by repeated intraperitoneal injections of a saline suspension of perfused, crushed rabbit kidney Streptococcus toxin was given concomitantly to most of the ducks as a non-specific antigen Details of these procedures will be described elsewhere

Nephrotoxic duck serum was injected intravenously into 19 rabbits In each animal after an incubation period of from five to ten days there was an abrupt onset of clinical nephritis—manifested by the presence in the urine of large amounts of albumin, many casts and fairly large numbers of leukocytes and red blood cells Specimens of urine obtained a day or two immediately after injection frequently

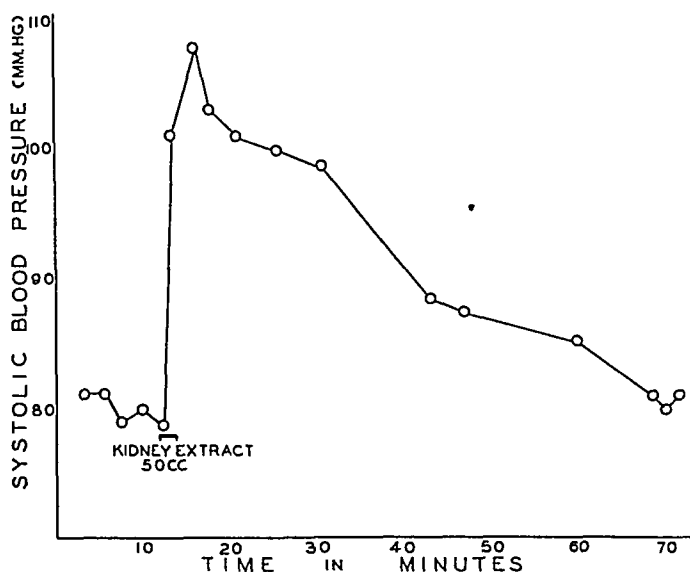


Fig 1—Systolic blood pressure response to 5 cc of kidney extract (prepared by a modification of the method described by Landis and associates<sup>23</sup>) as indicated by the method of determining blood pressure used in this study

contained small amounts of albumin, but this always disappeared before the onset of true nephritis

The clinical and pathologic characteristics of the nephritis in any particular animal depended on the potency and dose of the serum which had been injected In mild nephritis (occurring in 11 of the 19 rabbits) the health of the animal seemed generally unimpaired, and the blood nonprotein nitrogen did not rise above the normal range of from 25 to 50 mg per hundred cubic centimeters The urinary abnormalities gradually subsided to disappear entirely (except perhaps for a trace



of albumin in concentrated specimens of urine) in from fifty to one hundred days. The active period was considered to last from the onset of nephritis until the urine contained less than 250 mg of albumin per hundred cubic centimeters. Thereafter the animal was in the recovery period.

A rise in blood nonprotein nitrogen (at least two determinations of 60 mg or more per hundred cubic centimeters) was used as the criterion to differentiate severe nephritis (occurring in 8 of the 19 rabbits) from mild nephritis. Many of the rabbits with severe nephritis appeared ill, ate poorly and lost weight. One of the animals died in uremia sixteen days after the injection of the nephrotoxic serum. The urinary abnormalities were qualitatively the same in severe as in mild nephritis, but they were more marked in degree and longer in duration in the 7 animals which survived.

The nephritis which followed the first injection of nephrotoxic serum has been designated primary nephritis. In 8 rabbits in which all clinical evidences of the primary nephritis had disappeared (except in some instances a trace of albumin in the urine) nephritis was reprecipitated by again injecting nephrotoxic serum. In each instance, before the second injection of nephrotoxic serum the animal was desensitized by giving eight intravenous injections of normal duck serum in increasing doses, starting with 0.001 cc, during a two day period. This desensitization was necessary to avoid anaphylaxis. The reprecipitated nephritis differed little from the primary disease. In 3 of the 8 animals it was of the mild type. Severe nephritis developed in the other 5 animals, 3 of which died in uremia ten to nineteen days after the injection of nephrotoxin.

The clinical characteristics of the primary and the reprecipitated severe nephritis are illustrated in the case of rabbit 117.

This normal 1,900 Gm rabbit received normal duck serum in doses of 0.018 cc and 0.18 cc, respectively, on consecutive days in divided doses.<sup>29</sup> The following day 1.0 cc of nephrotoxic duck serum, lot X, was injected. Albumin first appeared in the urine on the seventh day and became massive the following day. The urinary sediment contained many hyaline casts and leukocytes and a few white cell casts, red cell casts and red blood cells. Nonprotein nitrogen rose from a control level of 37 mg to 110 mg per hundred cubic centimeters on the eleventh day and remained elevated for about four weeks. Albumin continued to be abundant in the urine for about two more weeks, but the casts and cells diminished in numbers. Thereafter urinary abnormalities gradually diminished and by the hundredth day the only remaining clinical evidence of the nephritis was a faint trace of albumin in concentrated urine specimens.

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<sup>29</sup> Although desensitization was actually necessary only in animals which had previously received injections, the administration of nephrotoxin was almost always preceded by a standard two day desensitization period so that the dosages in original and reinjection nephritis would be strictly comparable.

The animal was then desensitized and given 10 cc of nephrotoxin, lot X, exactly as before. No signs of anaphylaxis were observed. The ensuing nephritis was severe and characteristic of the reprecipitated disease. It differed little from the primary nephritis in the same animal. The incubation period was shorter, and the rise in nonprotein nitrogen was not quite as great. Urinary abnormalities and the course of the disease were essentially the same as had previously been observed. Blood pressure measurements in this animal are illustrated in figure 2.

Seven rabbits which had recovered from an attack of primary nephritis were desensitized in the usual manner and given injections of normal duck serum. Nephritis developed in 3 of these animals. One (rabbit 128) showed mild nephritis and was killed sixteen days after injection. One (rabbit 82) died in uremia at the end of thirty-six days.

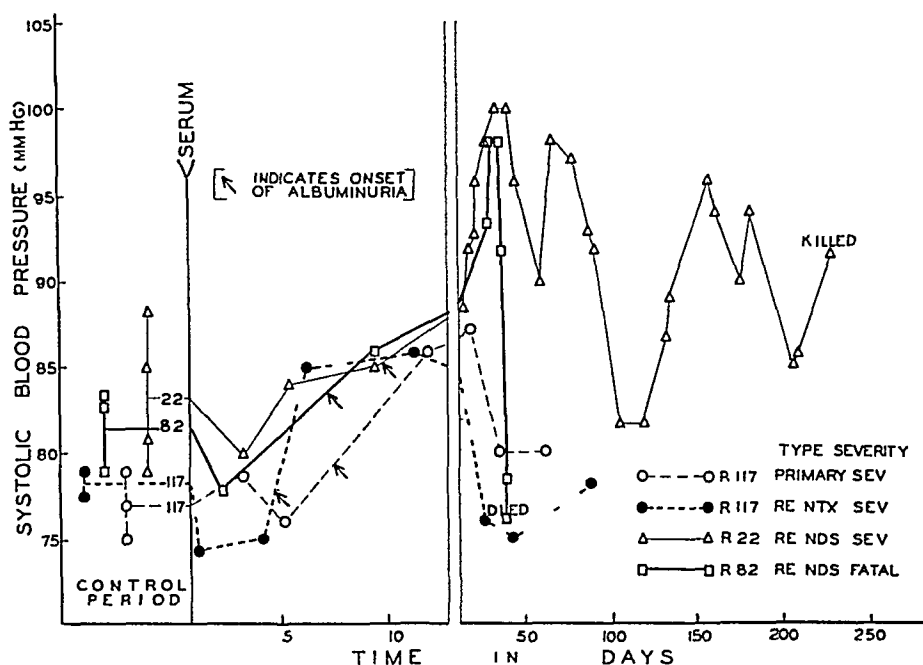


Fig 2—Systolic blood pressure in millimeters of mercury in 3 rabbits through four courses of nephritis. Each measurement is represented by a symbol (circle, triangle or square) and was derived from several readings. The abbreviations are as follows: R, rabbit, RE-NTX, reprecipitated nephritis, from injection of nephrotoxic duck serum, RE-NDS, reprecipitated nephritis, from injection of normal duck serum, SEV, severe.

One (rabbit 22) was killed two hundred and twenty-nine days after receiving the injection, while the clinical nephritis was still in the active stage. The urinary findings for these animals were essentially the same for the other nephritic animals. Red blood cells and red cell casts were not more numerous. The pathologic process seemed to be the same, although the histologic appearance of the kidneys was altered by the duration of the disease. In rabbit 22 the nonprotein nitrogen was consistently greater than 100 mg per hundred cubic centimeters for

TABLE 1—*Mean Systolic Blood Pressure of Each Rabbit by Type of Nephritis, Severity of Nephritis and Period of Disease*

Rabbit	Primary Nephritis				Reprecipitated Nephritis			
	Severity of Nephritis	Mean Systolic Blood Pressure (Mm of Mercury) with Number of Measurements* (in Parenthesis)			Severity of Nephritis	Mean Systolic Blood Pressure (Mm of Mercury) with Number of Measurements* (in Parenthesis)		
		Control Period	Incubation Period	Active Period		Control Period	Incubation Period	Active Period
17	Mild				Severe	77.3 (3)	66.0 (2)	81.8 (5)
21	Severe				Severe	80.3 (3)	71.0 (1)	81.5 (1)
22	Severe				Severe†	83.3 (1)	83.0 (3)	92.1 (24)
121	Mild	80.0 (3)		77.5 (2)	Mild	76.0 (2)	71.0 (2)	77.9 (4)
95	Mild	81.7 (3)	78.0 (2)	80.5 (4)				
230	Mild	76.0 (3)	74.5 (4)	75.5 (2)				
92	Mild	84.0 (3)		85.0 (3)	Mild	81.5 (2)	85.0 (1)	84.3 (3)
90	Mild	73.5 (2)	76.0 (2)	75.3 (3)				
93	Mild	82.7 (3)	78.5 (2)	84.8 (5)				
123	Mild	72.0 (2)	76.0 (2)	75.0 (2)				
91	Mild	84.5 (2)	89.0 (1)	90.0 (2)	Mild	85.5 (2)		91.0 (1)
34	Mild	78.7 (3)	82.0 (1)	85.8 (8)	Severe	79.0 (1)		87.8 (6)
140	Mild	73.7 (3)	73.0 (1)	83.0 (3)				
82	Severe	81.5 (4)		81.8 (4)	Severe†	81.7 (3)	77.0 (1)	94.5 (6)
26	Severe	77.0 (3)	76.5 (2)	77.7 (7)				
122	Severe	70.5 (2)	72.0 (2)	71.3 (3)	Severe	67.5 (2)	68.0 (1)	78.7 (3)
117	Severe	77.0 (3)	77.5 (2)	85.3 (3)	Severe	77.5 (2)	73.0 (3)	82.3 (3)
128	Severe	77.0 (2)	79.0 (2)	86.0 (3)	Mild†	78.0 (2)	80.0 (1)	78.5 (2)
35	Severe	75.0 (3)	77.0 (2)	84.5 (2)				

\* Each measurement was derived from several readings (see text)

† In this rabbit nephritis was reprecipitated by an injection of normal duck serum

over two hundred days, and the urine continued to show large amounts of albumin until death. Definite hypertension was present in rabbit 82 and in rabbit 22 (fig 2)

RESULTS <sup>30</sup>

To determine the trend of blood pressure in the nephritic rabbits it was first necessary to ascertain the degree of variation in blood pressure from measurement to measurement in the normal animal. To obtain this information, the average of the blood pressure measurements for each animal in the control period was determined. The standard deviation of the individual blood pressure measurements from the respective averages of such measurements was then computed. The

TABLE 2—*Differences in Mean Systolic Blood Pressures from the Control Period to the Period of Active Nephritis*

Primary Nephritis			Reprecipitated Nephritis		
Rabbit	Severity of Nephritis	Change, Mm Hg	Rabbit	Severity of Nephritis	Change, Mm Hg
121	Mild	-2.5	92	Mild	-0.2
95	Mild	-1.2	128*	Mild	+0.5
230	Mild	-0.5	121	Mild	+1.0
92	Mild	+1.0	91	Mild	+5.5
90	Mild	+1.8			
93	Mild	+2.1	21	Severe	+1.2
123	Mild	+3.0	17	Severe	+4.5
91	Mild	+5.5	117	Severe	+4.8
34	Mild	+7.1	34	Severe	+8.8
140	Mild	+9.3	22*	Severe	+8.8
			122	Severe	+11.2
82	Severe	+0.3	82*	Severe	+12.8
26	Severe	+0.7			
122	Severe	+3.8			
117	Severe	+8.3			
128	Severe	+9.0			
35	Severe	+9.5			

\* In this rabbit nephritis was reprecipitated by an injection of normal duck serum

resulting figure indicated not only the actual variations in blood pressure from day to day in the animals but also the degree of accuracy of the method used to determine the blood pressure. It was found that the standard deviation of measurements from the mean was 1.97 mm of mercury in the control period. Thus significance could probably be attached to any measurement figure varying more than 3.94 mm of mercury from the mean of the control period.

By a similar process of computation it was found that the variation from the mean was slightly increased (2.72 mm) in the incubation period and further increased (3.30 mm) in the active period and that in the recovery period the variability of measurements decreased to

30 The statistical analysis was prepared under the direction of Dr. E. L. Crosby Jr., of the statistical departments of the Johns Hopkins University and Hospital.

164 mm These figures indicate that the blood pressure was more labile in the incubation and active periods of the nephritis than in the control period

The average of the measurements for each animal in each period is shown in table 1

It is evident from these figures that no significant rise in blood pressure occurred during the incubation period of the disease and that in most instances the figures for the recovery period closely approximated those for the control period The differences in average blood pressures in the control period from those of the active period are shown in table 2

It is apparent that in many animals the level of blood pressure was not significantly higher in the active than in the control period Slight elevations were more frequently present in the severely nephritic rabbits than in those in which the disease was mild The general trend was about the same in primary nephritis as in nephritis reprecipitated by nephrotoxic duck serum An elevation in blood pressure was most clearly apparent in rabbits 22 and 82, in which the nephritis was reprecipitated by an injection of normal duck serum From a detailed statistical analysis it is possible to state that the small rise in blood pressure observed in some of these animals could not have occurred as a result of chance alone

#### SUMMARY

Experimental nephritis has been produced in rabbits by injecting anti-rabbit-kidney duck serum Blood pressure was determined in 19 rabbits through thirty courses of nephritis Blood pressure was more labile during the active period of nephritis than in the control period No significant elevation in blood pressure occurred between the time of injection of the nephrotoxic serum and the appearance of the clinical nephritis A small rise in blood pressure occurred during the active period of the nephritis in some of the animals, but this was not an invariable manifestation of the disease These slight elevations of blood pressure were more frequently observed in severely nephritic animals than in those in which the disease was mild

Changes in blood pressure were essentially the same in the primary attack of nephritis as in the nephritis subsequently induced by injection of nephrotoxic duck serum

A moderate elevation in blood pressure was most clearly apparent in 2 rabbits in which severe nephritis was reprecipitated by injection of normal duck serum It persisted more than two hundred days in 1, a rabbit in which the nephritis had become chronic

# PATHOLOGIC AND HISTOLOGIC CHANGES FOLLOWING ORAL ADMINISTRATION OF SULFAPYRIDINE

WITH A SHORT NOTE ON SODIUM SULFAPYRIDINE

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AND

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Recently we reported the occurrence of urinary concretions in the rat, rabbit and monkey, consisting mainly of acetyl sulfapyridine, following oral administration of sulfapyridine (2-[paraaminobenzenesulfonamido]-pyridine)<sup>1</sup> Similar findings were reported by Gross, Cooper and Lewis<sup>2</sup> in experimental studies on rats Previous to the foregoing reports, Oakley<sup>3</sup> in a short note on poisoning in mice due to "prontosil" (form not specified) reported the presence of "prontosil" crystals in the urinary bladder and gallbladder as well as concretions in the straight and convoluted tubules Stewart, Rourke and Allen<sup>4</sup> observed the appearance of sulfanilamide crystals in the urine of patients and suggested the possibility of stone formation in the urinary tract Laurence<sup>5</sup> was the first to report a case in which a patient experienced sharp pain in the lower right quadrant of the abdomen followed by gross hematuria, after administration of sulfapyridine and attributed this to the acetylated derivative One of us (W A ) has observed 2 similar cases in which the findings were highly, but not conclusively, suggestive of urolith effects The presence of crystals in the urine of patients receiving sulfapyridine has already been reported by Stokinger<sup>6</sup>

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From the Laboratories of the Newark Beth Israel Hospital and the Merck Institute of Therapeutic Research

1 Antopol, W, and Robinson, H Proc Soc Exper Biol & Med **40** 428, 1939 Molitor, H, and Robinson, H Arch internat de pharmacodyn et de therap **62** 281, 1939

2 Gross, P, Cooper, F B, and Lewis, M Proc Soc Exper Biol & Med **40** 448, 1939

3 Oakley, C L Biochem J **31** 729, 1937

4 Stewart, J M, Rourke, G M, and Allen, J C J A M A **110** 1885, 1938

5 Laurence, E A, in International Review of Recent Advances in Medicine, Washington, D C, Washington Institute of Medicine, 1939, vol 5, p 48

6 Stokinger, H E Proc Soc Exper Biol & Med **40** 61, 1939

In this paper we wish to present a report of pathologic and histologic changes following oral administration of sulfapyridine. A short description of the effects of sodium sulfapyridine is also included.

The animals used in these experiments were maintained on a balanced diet with sufficient water. Certain details of the experimental technic used in this investigation have been reported by Molitor and Robinson<sup>7</sup> in a study concerning the toxicity of sulfanilamide.

#### EXPERIMENTAL RESULTS

Concretions were observed after the administration of a large single dose of sulfapyridine, but the results were more striking after repeated dosing on successive days. The dosage at which uroliths were formed varied greatly in different species but to a much lesser degree in individuals of the same species. Thus, after sulfapyridine had been administered orally, concretions were frequently found in rabbits given 10 to 15 Gm per kilogram, in rats given 5 Gm per kilogram and in monkeys fed 0.25 Gm per kilogram. When smaller doses were given, the individual variation in a given species was increased. In mice and dogs urolith formation was not observed even after the feeding of doses as large as 20 Gm per kilogram. It is interesting to note that the latter species acetylate the compound very little.

In the course of this investigation approximately 400 mice, 320 rats, 42 rabbits, 16 dogs and 48 monkeys were used.

#### PATHOLOGIC OBSERVATIONS

With the dosage necessary for each species, the pathologic results were essentially alike in the rat, rabbit and monkey, and therefore a single description will suffice for all of these species.

The urolith formation was often unilateral, occurring more frequently on the right side. If the animal was put to death approximately twenty-four hours after the administration of the drug, aggregates of these crystals were often found in the ureter, especially at the level of the bony pelvic brim. After five to ten days of feeding the concretions were frequently found near the ureterovesical junction (fig 1) or at times in the bladder. The calculi were soft and very friable, breaking up into minute fragments on slight pressure. Most of the concretions were irregularly ovoid but at times were elongated and formed a cast of the ureter. In animals killed during the early part of the feeding period the ureter was dilated and thinned out. Later it was indurated and in some instances markedly hemorrhagic. The kidney became edematous and enlarged to one and a half to two and a half times its

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<sup>7</sup> Molitor, H., and Robinson, H. *J. Pharmacol. & Exper. Therap.* **65**: 405, 1939.

normal size (fig 1) At this time the kidney and ureters were found to be intimately bound with the periureteral tissues Bloody urine was found within the dilated portion of the renal pelvis and ureter In some instances an amorphous aggregate of crystals and fibrin completely filled the renal pelvis and extended upward into the papillary ducts of the kidney (fig 2) but did not extend downward beyond the ureteropelvic junction In these circumstances the ureter appeared normal

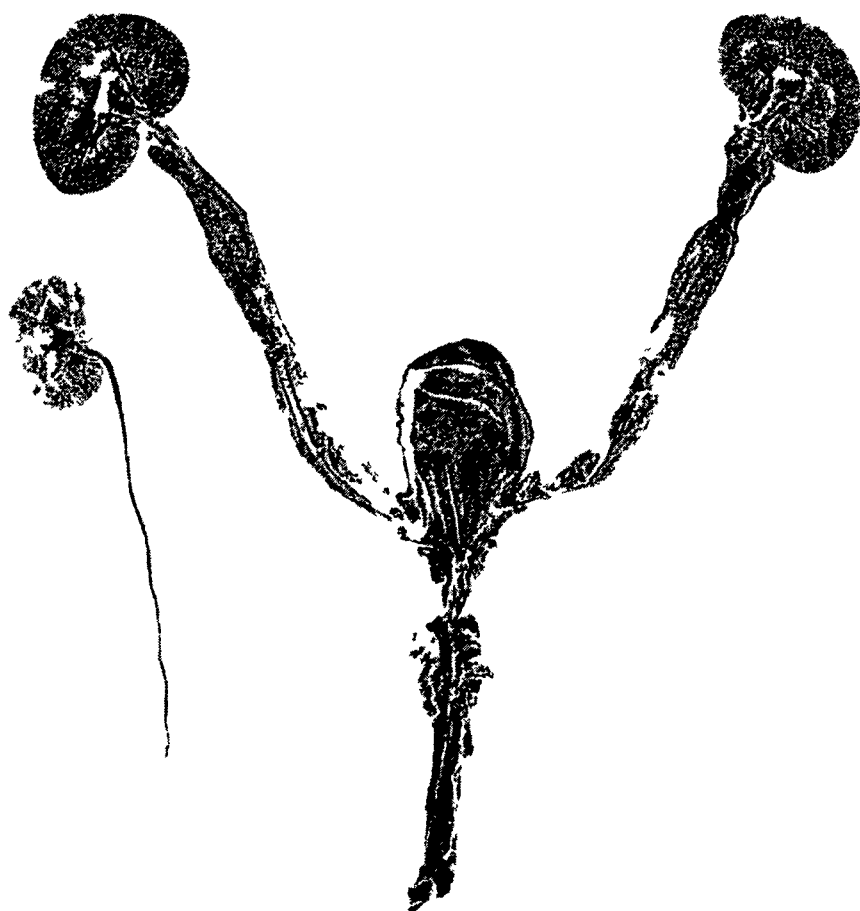


Fig 1—Urinary system from a monkey which received 14 Gm of sulfapyridine per kilogram for five days and died on the fifth day The blood urea nitrogen was 82 mg per hundred cubic centimeters Calculi were found at the ureterovesical junction, with hydroureter, hydronephrosis and edematous enlargement of the kidney Compare this with the normal kidney and ureter at the left

The bladder was often edematous, especially in the region of the trigone The ureterovesical orifices were prominent because of the lipping and congestion of the surrounding tissue



Urea nitrogen was determined by the method of Van Slyke on the blood of the monkeys. All showed increased retention, ranging from a slightly elevated level to 100 mg in 100 cc of blood.

#### HISTOLOGIC OBSERVATIONS

*Kidney (Rhesus Monkey)* —The kidney revealed degeneration varying from mild to most severe, especially in the proximal convoluted tubules. With larger doses, the proximal and distal convoluted tubules could not be differentiated from each other. The cytoplasm of the cells forming these tubules was coarsely granular and occasionally vacuolated. Hyaline droplet degeneration was rare. The lumens of the

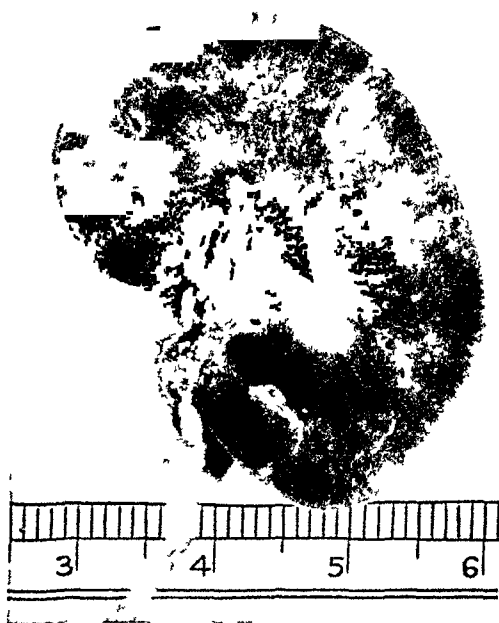


Fig 2—Kidney of a monkey receiving 5 Gm of sulfapyridine per kilogram for six days. The blood urea nitrogen was 80 mg per hundred cubic centimeters. Amorphous aggregates of crystals and fibrin completely fill the renal pelvis and extend into the papillary ducts.

tubules contained globoid eosinophilic bodies. The loops of Henle were markedly distended and contained, in addition to a few globular elements similar to those found in the convoluted tubule, a coagulum which was eosinophilic with hematoxylin and eosin and blue with azocarmine. In some instances the cells lining the parietal layers of Bowman's capsule were covered by cytoplasm in a cuboid form, in which a nucleus was rarely observed, when one was present, the cell closely resembled a cell of the proximal convoluted tubules (fig 3 A). These cells stained red with azocarmine, and transitions between these and the globular red-

staining bodies found in Bowman's space were noted. Apparently these are identical with the globoid bodies described in the tubular lumens. With azocarmine the glomeruli showed marked thickening of the basement membrane of the glomerular tuft (fig 3 *B*). When doses of sulfapyridine insufficient to produce concretions were administered, the aforementioned degenerative changes were also found, though usually not as pronounced. Following excessive dosage, the glomeruli were markedly distended and contained, in addition to the cell debris, a coagulum similar to that found in the loops of Henle. The corresponding glomerular tufts were compressed, and the capillaries were collapsed and bloodless (fig 3 *C*).

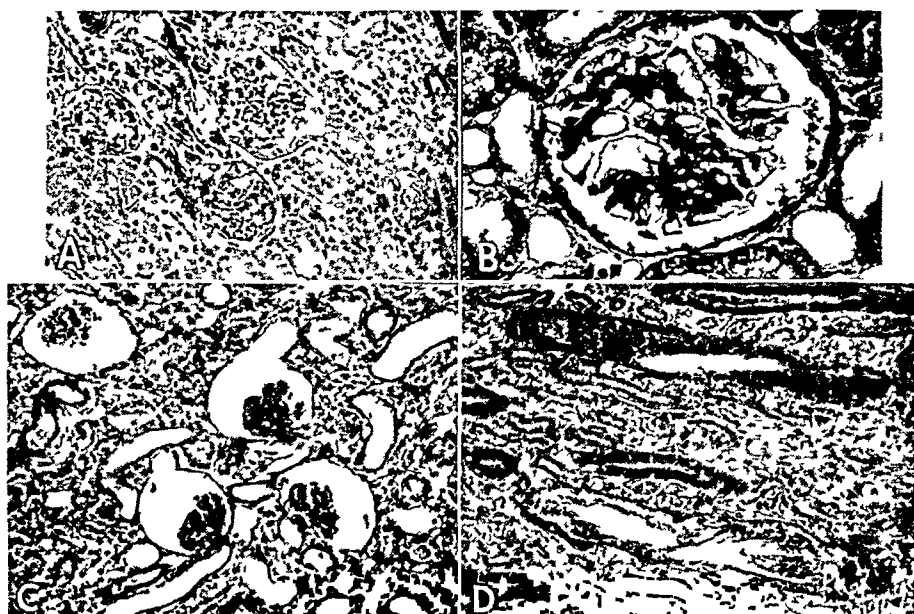


Fig 3—*A*, parietal layer of Bowman's capsule covered by cytoplasm in cuboid form, apparently in continuity with the epithelium of the proximal convoluted tubule,  $\times 90$ . *B*, azocarmine stain showing thickened basement membrane of the glomerular tuft,  $\times 250$ . Note globoid eosinophilic bodies in Bowman's space. *C*, distended glomerular spaces with compression of glomerular tuft,  $\times 90$ . *D*, fibrin scaffolding in papillary ducts showing spaces from which crystals were dissolved out in preparation of section,  $\times 90$ .

The collecting tubules showed only slight to moderate distention. The lumens of the papillary ducts were filled with coagulum, and only a few contained an appreciable number of the globoid bodies described. Other collecting tubules and papillary ducts contained coagulum and fibrin with clefts corresponding to spaces in which crystals had been present (fig 3 *D*). This was most frequently observed when the renal pelvis was filled with the crystals and fibrin. Many of the tubules con-

tained and were surrounded by a mantle of polymorphonuclear leukocytes, and rarely a foreign body type of giant cell was found between the tubules

Pyelonephritis and purulent periureteritis and peripyelitis were also observed

If the animals were fed over a long period or had been permitted to survive for long periods after ample dosage, calcium casts were found in the tubules (fig 4 *A*)

In the cases in which calculi were present within the ureter, the ureter and the renal pelvis showed from slight to extremely pronounced

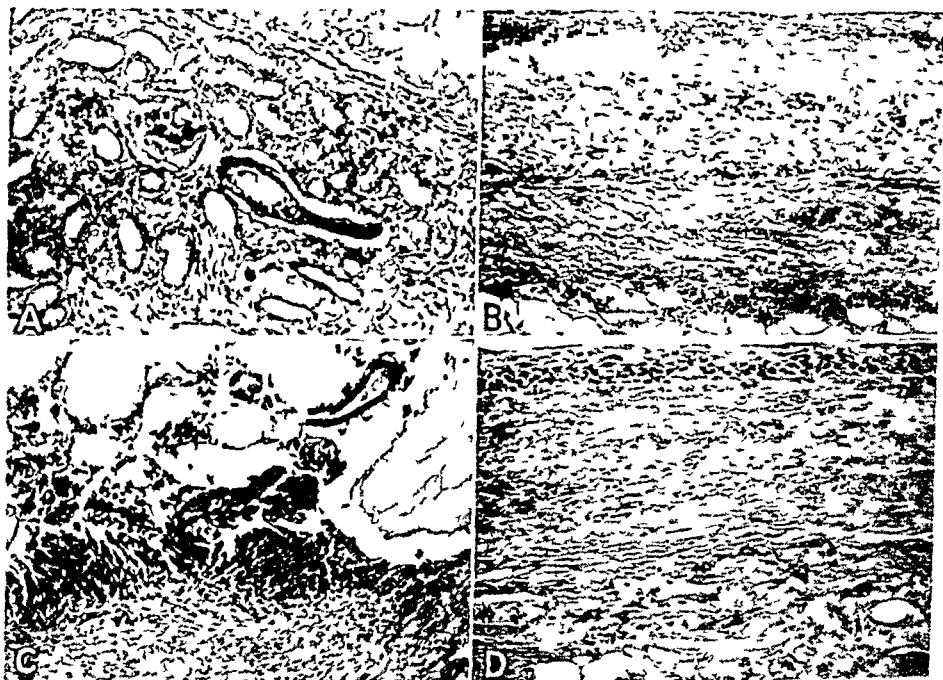


Fig 4—*A*, calcification of tubules,  $\times 90$  *B*, ureter showing edema, slight hemorrhage and a scattering of polymorphonuclear leukocytes,  $\times 90$  *C*, fibrin scaffolding in pelvis with clefts which contained crystals,  $\times 90$  *D*, edematous and hemorrhagic ureter

edema of the lamina propria, with a moderate diffuse scattering of polymorphonuclear leukocytes (fig 4 *B*) In isolated instances a phlegmon was found The epithelium for the most part was intact, but an occasional erosion was observed

In those cases in which the renal pelvis was found to be filled with an amorphous collection of fibrin and smaller calculi, the pelvis contained fibrinous material with clear clefts, which probably had the same significance as those described in the papillary ducts and collecting tubules (fig 4 *C*) In places the transitional epithelium was absent, and in

these locations the fibrin rested directly on the lamina propria. The blood vessels in these regions showed extreme dilatation.

At times there occurred extremely hemorrhagic ureteritis and pyelitis, far in excess of that which might be expected from the presence of the corresponding calculus (fig 4 *D*). Occasionally thrombi were encountered in the veins of the kidney. In some instances obliterating lesions (fig 5 *B*) were found within lumens of veins which were not in close proximity to any of the inflammatory zones. Intraureteral hemorrhage was at times excessive, and in 2 cases the clotted blood caused urinary obstruction (fig 5 *A*).

In some instances, after repeated feeding with doses insufficient to produce concretions the kidneys were edematous and revealed the glomerular and tubular changes described except that no "crystal casts" were found in the tubules. Because of this and the fact that, as men-

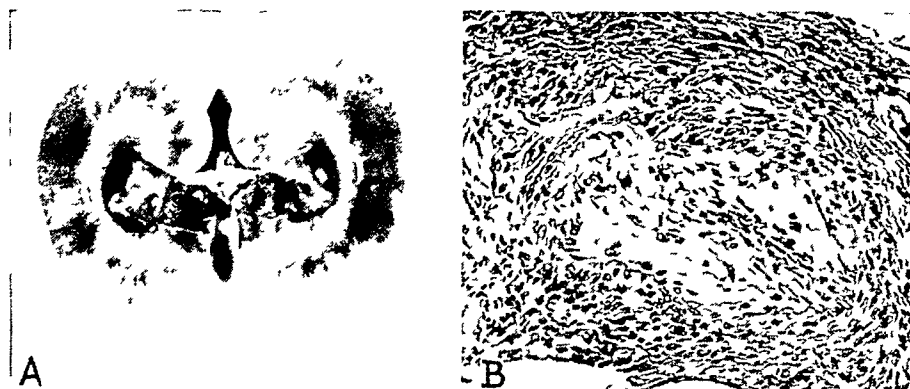


Fig 5—*A*, kidneys of a monkey receiving 5 mg of sulfapyridine per kilogram for seven days, which was killed twenty-three days after the last feeding. The blood urea nitrogen was 11 mg per hundred cubic centimeters. Note blood clot causing obstruction in the renal pelvis. *B*, obliterating endophlebitis,  $\times 130$ .

tioned earlier, the hemorrhagic reaction may be extreme, the possibility must also be entertained that the drug may first produce parenchymal damage, and that subsequent to this the acetylated compound is precipitated from solution.

There are experimental data suggesting that the obstructing crystalline mass can either be redissolved or washed out. In a series of 5 monkeys, all of which received 4 Gm of sulfapyridine per kilogram for ten days, 4 animals which were killed on the last day of feeding showed urolithiasis, while in the fifth, which was put to death seventy-nine days after the discontinuation of this treatment, no concretions were found. This animal exhibited, as evidence of transient urinary obstruction, slight thickening and dilatation of the ureters and pelvis. There were perirenal, peripelvic and periureteral adhesions, and the renal cap-

sule could not be stripped without tearing the renal parenchyma. The kidney showed scattered extensive collections of round cells, particularly in the subcapsular region (fig 6 *A*). Many of the tubules were filled with densely eosinophilic coagulum. Most of the glomeruli showed no appreciable damage. However, in the areas where the round cell infiltration was present, some were bloodless, others showed marked increase of cytoplasm, while others were undergoing fibrosis (fig 6 *B* and *C*). Bowman's capsule was occasionally thickened. One small vein showed recanalization. There was extensive round cell infiltration in the ureter and pelvis, and in places the epithelium dipped into the lamina propria (fig 6 *D*).



Fig 6—Sections of kidney of a monkey receiving 4 Gm of sulfapyridine per kilogram for ten days, which was put to death seventy-nine days after the last feeding,  $\times 90$ . No calculi were found. *A*, extensive round cell collections in the subcapsular region. The capsule could not be stripped without tearing the kidney substance. Note the dilated tubules filled with eosinophilic coagulum. *B* and *C*, areas showing round cell infiltration. Many glomeruli are bloodless, with increase in cytoplasm. *D*, subepithelial round cell infiltration in pelvis.

The uroliths ordinarily permit penetration by roentgen rays and therefore do not cast a shadow. However, calcium may be deposited about these concretions, which act as a nucleus, in which case the shell may become roentgen ray opaque.

*Adrenal*—In the rat the adrenal was enlarged, and the cortex appeared gray. Histologically, the fascicular layer was markedly widened.

and the cytoplasm in this area was deeply eosinophilic and in many instances showed no vacuolation and contained little lipid. In the monkey, in the early stage the changes were similar to those in the rat but not as pronounced. Prolonged feeding did not result in greater changes.

There were occasionally calcific areas in the adrenal, but these were also encountered in the normal controls.

*Liver*—At times, especially in the monkey, the liver showed separation of the capillaries from the liver cord cells, with granular material in the intervening spaces—similar to the "serous hepatitis" of Rossle-Eppinger. With Best's carmine stain, the liver cells were diffusely filled with glycogen.

*Other Organs*—The stomach often showed marked congestion with an increase in mucous secretion. The heart did not show any significant changes. The cells lining the acini of the thyroid were cuboid. The colloid, especially near the cell periphery, was pale staining, suggesting mild hyperplasia. The bone marrow showed an increase in polymorphonuclear leukocytes. The response of the marrow will be reported later in collaboration with L. Goldman and W. Sampson. The spleen showed considerable deposition of iron, especially marked in the perifollicular zone. Erythrophagocytosis was not uncommon in the pulp. The pulp usually showed marked congestion. Lymphoid hyperplasia was variable. The lymph nodes were usually hyperplastic and showed marked proliferation of the lining cells of the sinuses.

#### COMMENT

From the observations described one cannot conclude definitely whether the precipitation of the acetylated sulfapyridine in the urine is always primary or whether it is dependent on initial degenerative changes in the renal parenchyma or on a combination of both. Since the degenerative changes are found without urolith formation in some instances, one may safely assume that the former changes are not dependent on the formation of concentrations. The possibility remains that the formation of uroliths may at times be initiated by the degenerative renal or vascular changes, on the other hand, it may be quite independent of these changes.

With the oral administration of sodium sulfapyridine, smaller doses were necessary for urolith formation in the rat, rabbit and monkey. Histologically the degenerative changes in the kidney were more pronounced than those found in animals fed sulfapyridine. In addition the mucous secretion of the stomach was very striking, and in isolated

instances the rabbit showed acute diffuse gastritis. When this drug was administered rectally, a hemorrhagic reaction with some necrosis of the mucosa was frequently encountered.

#### SUMMARY

Urolithiasis occurs after the feeding of sulfapyridine to monkeys, rabbits and rats. The concretions can be either redissolved or washed out. It cannot be ascertained whether the formation of the urolith is always an independent precipitation process or whether it is at times dependent on primary degenerative or vascular changes in the kidney.

# PLASTIC STUDIES IN ABNORMAL RENAL ARCHITECTURE

## V THE PARENCHYMAL ALTERATIONS IN EXPERIMENTAL HYDRONEPHROSIS

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In his classic study of the problem of hydronephrosis Ponfick,<sup>1</sup> recognizing the inadequacy of the method of histologic section, summarized the difficulties that he encountered in the identification of structures and in the interpretation of the topographic interrelations of the altered renal parenchyma in the following words

Denn infolge der tiefgreifenden Umwälzungen, die sich an den Tubulis verschiedenster Art vollzogen haben, sind deren Eigentümlichkeiten nachgerade dermassen verwischt, dass man wohl im Zweifel bleiben kann, ob man contorti oder recti, vielleicht sogar Henlesche Schleifen vor sich habe (For, owing to the profound changes which have taken place in the various types of tubuli, their characteristics are blurred to such an extent that one may remain in doubt whether one has to do with convoluted or with straight tubules or even with Henle's loops )

As has been demonstrated by Oliver and his co-workers,<sup>2</sup> the method of microdissection particularly favors the elucidation of such problems, and on his suggestion the method has been applied to the study of the effect of ureteral ligation on the rabbit kidney In the discussion that follows it will become apparent that many of the assumptions that have been made concerning histologic pictures in hydronephrosis are without support when viewed in the reality of three dimensions

That the technic of microdissection previously had been applied to this problem<sup>3</sup> appeared not to contraindicate its further use, as one does not find in the extensive subjective interpretations of this worker's fragmentary presentations the essential value of the method, which is the demonstration of actual objects in structural continuity and their topographic relations

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From the Department of Pathology of the Long Island College of Medicine,  
the Hoagland Laboratory

1 Ponfick, E Beitr z path Anat u z allg Path **49** 127, 1910

2 (a) Oliver, J, and Lund, E M Arch Path **15** 755, 1933 (b) Oliver, J, and Luey, A S ibid **18** 777, 1934, **19** 1, 1935 (c) Loomis, D ibid **22** 435, 1936 (d) Oliver, J The Architecture of the Kidney in Chronic Bright's Disease, New York, Paul B Hoeber, Inc, 1939

3 Johnson, C H J Urol **27** 279, 1932



## MATERIAL AND METHODS

Rabbits were the animals of choice because of the simplified form of their kidneys and because normal rabbit kidneys have been studied intensively by the method of microdissection by Peter<sup>4</sup> The animals used included both males and females and were usually between 6 months and 1 year old Under ether anesthesia their left ureters were ligated and cut about 1 cm below the ureteropelvic junction without interference with the renal vessels Fourteen animals were put to death at intervals of from one to two hundred and thirty-one days Six additional animals, about 6 months old, were similarly treated and put to death at intervals

*Data on Experiments*

Rabbit	Weight of Rabbit, Gm	Duration of Obstruction, Days	Weight of Kidney, Gm	Measurements of Kidney	Volume of Pelvic Fluid, Cc
6	2,360	1	R 82 L 146	34 × 25 × 20 41 × 29 × 25	4
20	1,870	3	R 80 L 175	35 × 25 × 20 45 × 32 × 28	6
21	2,270	6	R 89 L 269	35 × 25 × 20 50 × 35 × 30	8
14	2,380	10	R 92 L 251	34 × 24 × 21 50 × 37 × 28	96
7	2,210	14	R 78 L 236	33 × 22 × 21 42 × 27 × 24	10
15	2,050	21	R 70 L 290	30 × 20 × 13 40 × 26 × 21	12
16	2,300	25	R 74 L 342	33 × 20 × 13 47 × 31 × 22	184
17	2,340	36	R 81 L 458	32 × 23 × 15 56 × 40 × 33	35
8	2,450	46	R 829 L 580	36 × 24 × 16 63 × 46 × 42	46
19	2,750	61	R 93 L 544	40 × 25 × 20 60 × 40 × 40	40
9	2,400	90	R 80 L 210	38 × 25 × 20 45 × 38 × 29	15
13	2,470	114	R 84 L 173	37 × 24 × 20 45 × 36 × 30	12
10	2,900	163	R 84 L 141	36 × 25 × 21 40 × 32 × 26	10
11	2,550	231	R 81 L 85	35 × 25 × 20 32 × 24 × 23	6

of from three to six weeks Another animal was excluded from the series because a staphylococcic infection of the obstructed kidney was discovered after five weeks, but the other hydronephrotic kidneys remained free from infection

After the kidneys had been weighed and measured and the pelvic fluid removed (table), the organs were sectioned longitudinally and then cut transversely, the first blocks being taken through the center of the papillary area Blocks were fixed in Zenker's fluid for routine hematoxylin and eosin stains and for iron stains, in solution of formaldehyde U S P for fat stains and in Kolster's fluid for the Altmann stain for mitochondria The remainder of the organ was placed in solution of formaldehyde until it could be dissected

4 Peter, K Untersuchungen über Bau und Entwicklung der Niere, Jena, Gustav Fischer, 1927

Thin blocks of tissue then were cut through the papilla adjacent to that used for the routine microscopic sections. These blocks were allowed to stand in concentrated hydrochloric acid at room temperature until softened sufficiently for dissection, they were then washed in several changes of water and placed in distilled water in a Stender dish. The tissue was then dissected under a binocular microscope with the aid of needles. The nephrons were examined at magnifications of 33 and 100 while supported in water and were photographed stereographically at a magnification of 8 times. From the plates, transparencies were prepared and mounted for study in the stereoscope, and from these the final drawings of figures 10 to 42 were made. The location of each specimen photographed, together with notes on the topographic relations, were recorded at the time on an outline drawing of the block of tissue being examined, and from these data, supplemented by the study of histologic sections, the topographic arrangements of figures 43 to 54 were prepared. For purposes of description the transverse sections of the kidney are divided into central, intermediate and lateral areas. It will be seen that this division is not arbitrary but is made in accordance with distinctive structural alterations developing within these three zones.

#### GROSS STRUCTURAL CHANGES

In general, the macroscopic changes in renal architecture resulting from ureteral ligation are well known, however, several features of the gross findings merit particular attention.

As early as three days after ligation of the ureter the papilla of the rabbit kidney is literally torn apart (fig 1), and shortly thereafter the ducts of Bellini are no longer to be found. At the site of the papilla there develops an irregular oval depression, and from its margins radiate the interrupted collecting tubules (fig 2). With the advance of the lesion the depression becomes larger as the tubules are increasingly drawn apart by the accumulation of the pelvic fluid. With the passage of time these interrupted tubules become enclosed by connective tissue and covered by epithelium, so that in the twenty-eight day specimen the continuity of the pelvic and tubular epithelium has been restored, and in the sixty-one day specimen the collecting tubules are found separated from the pelvis by connective tissue and the pelvis is lined by an uninterrupted sheet of epithelial cells (fig 3).

The parenchymal weights of obstructed kidneys increase for periods up to and including sixty-one days and beyond this interval decrease progressively to reach a figure below the original (table). The rate of gain is variable. It is greatest in the first few weeks of obstruction. This finding corresponds with that of Ponfick<sup>1</sup> and, as he described seems due to congestion, edema and accumulation of fluid in dilated tubules.

In the first few weeks, when the secretory capacity of the parenchyma may reasonably be assumed to be best maintained, the rate of accumulation of pelvic fluid is relatively slow and inconstant. The fluid is yellowish or only slightly blood tinged, is moderately turbid and



Fig 1 (rabbit 20) —Three days' obstruction

Fig 2 (rabbit 8) —Forty-six days' obstruction

Fig 3 (rabbit 9) —Ninety days' obstruction The three figures show normal and hydronephrotic kidneys and longitudinal sections of the latter showing (1) the variation in volume of the organs, (2) flattening, splitting and tearing of the papilla and (3) newly proliferated uninterrupted pelvic epithelium covering the site of the obliterated papilla

contains small numbers of desquamated epithelial cells, leukocytes, hyaline casts and considerable albumin. Between the fourth and seventh week of ureteral occlusion there is a rapid increase in volume of pelvic fluid, and it changes in character, becoming frankly bloody.

On sedimentation, one third of the pelvic fluid of the thirty-six day specimen is found to consist of packed erythrocytes. The forty-six day specimen yields the maximum volume of pelvic fluid, and this contains a slightly increased proportion of packed erythrocytes. The blood in these instances is obviously fresh and becomes correspondingly less so in later specimens. There is only a slightly decreased volume of pelvic fluid in the sixty-one day specimen, but later the fluid gradually decreases in volume (table) and becomes thinner and lighter in color, although it remains somewhat turbid.

#### PARENCHYMAL ALTERATIONS

*Glomeruli*—(a) *Microscopic Sections* Owing to compression of the capillary tuft, Bowman's space is enlarged and frequently contains a slight amount of coagulated fluid and rarely an occasional erythrocyte. In some examples the tuft (figs 4 to 9) shows cleftlike spaces between adjacent capillary loops, and throughout the series the latter are relatively bloodless. In the forty-six day specimen the parietal layer of Bowman's membrane is slightly thickened, and this change is somewhat more marked in later stages (fig 9), but connective tissue proliferation and hyalinization within the tuft are not seen until two hundred and thirty-one days of obstruction and then are inconspicuous in most glomeruli (fig 9). There is no appreciable increase in the external diameter of glomeruli save in certain cystic dilatations of the space of Bowman to be described later. These occur in the forty-six, sixty-one and ninety day specimens, and histologically the glomeruli present no structural peculiarities save their increase in size and the thickening of Bowman's membrane mentioned.

(b) *Microdissection* Save in rare instances, in later stages the volume of the glomeruli is not increased, on the contrary, reduction in glomerular volume is evident in twenty-four hours and in general is maintained throughout the period of the experiments. Although in the latest stage a further reduction in volume is apparent, there is nothing to suggest the complete disappearance of glomeruli during the two hundred and thirty-one day interval of this study.

In the early stages variability in size and shape is greater than normal, and although examples of concomitant decrease in size of glomeruli and their proximal convoluted tubules are found, many of the atrophied proximal convoluted tubules too minute for dissection possess glomeruli as large or even larger than those associated with the best preserved proximal convoluted tubules of the same specimen (figs 23 and 26).



Fig 4 (rabbit 20) —Three days' obstruction Dilatation of tubular lumens and flattening of epithelial cells in the inner part of the cortex of the central area Magnification, 150

Fig 5 (rabbit 14) —Ten days' obstruction Increasing dilatation of tubular lumens with beginning distention of collecting tubules and of distal convoluted tubules and variable atrophy of proximal convolutions in the inner part of the cortex of the central area Magnification, 150

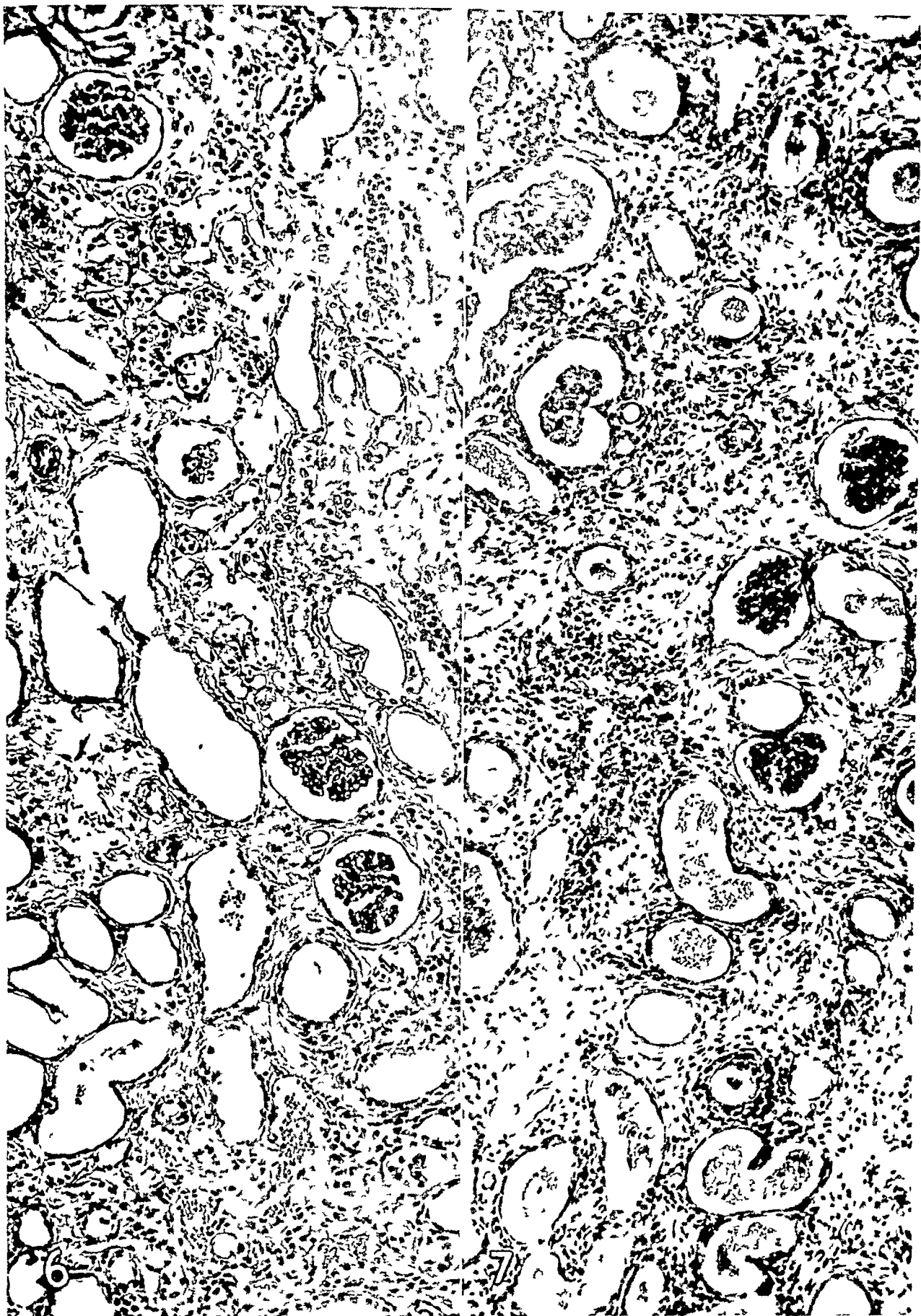


Fig 6 (rabbit 16) —Twenty-eight days' obstruction Increased interstitial change and epithelial atrophy in the cortex of the central area The dilated tubules are distal convoluted tubules and collecting tubules Note the repeated cuts across one of the few identifiable proximal convoluted tubules just below the uppermost glomerulus Magnification, 150

Fig 7 (rabbit 17) —Thirty-six days' obstruction The epithelial atrophy, leukocytic infiltration and connective tissue proliferation are more marked in the cortex of the central area Note the blood accumulated in tubules known from dissection to be distal convoluted tubules Magnification, 150

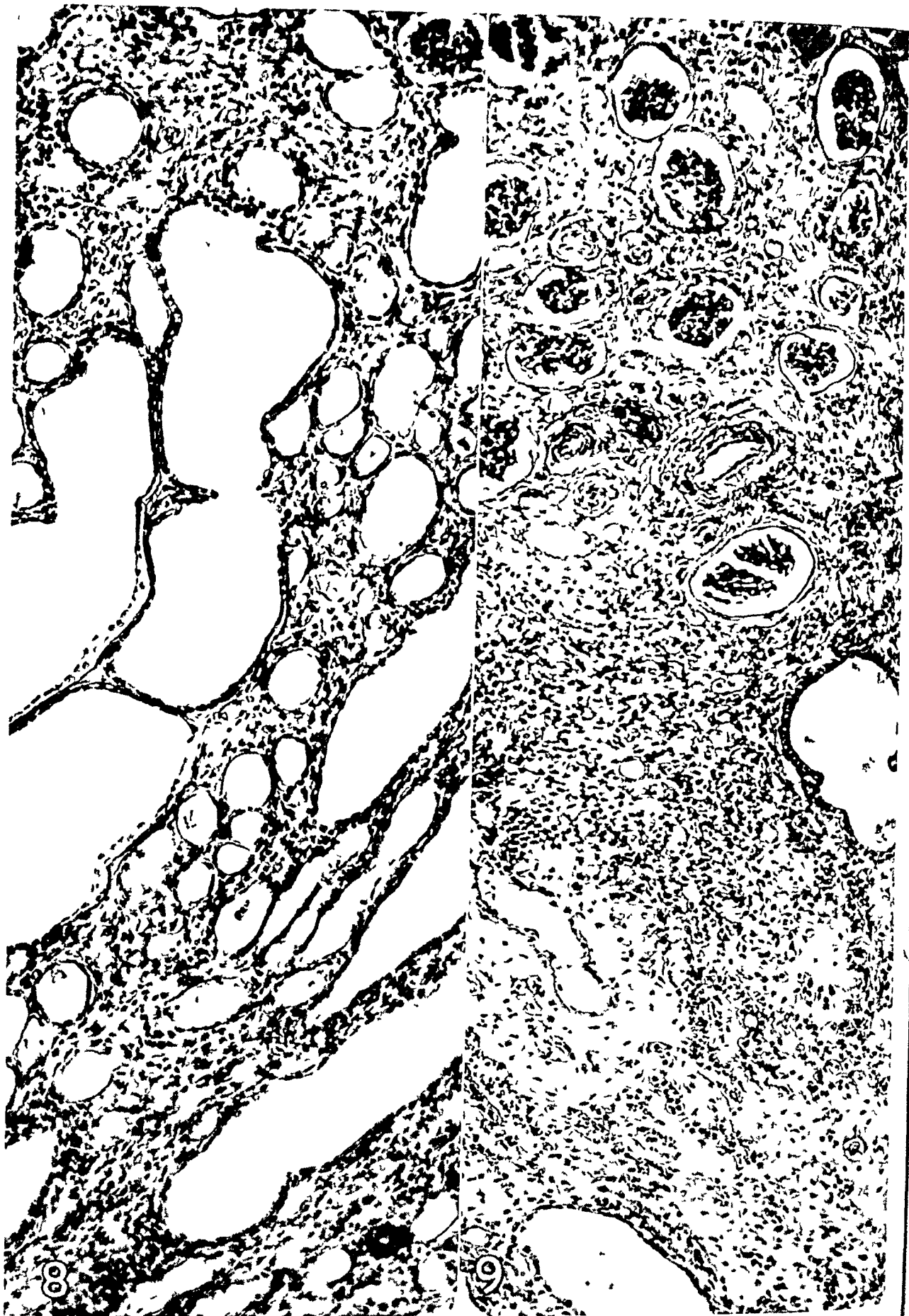


Fig 8 (rabbit 17) —Thirty-six days' obstruction Interstitial fibrosis, leukocytic infiltration and dilatation of collecting tubules in the outer portion of the medulla of the central area These collecting tubules are narrowed at the corticomedullary boundary Magnification, 150

Fig 9 (rabbit 11) —Two hundred and thirty-one days' obstruction The connective tissue between the concentrated glomeruli is infiltrated by leukocytes and is practically devoid of identifiable tubular remnants The capillary tufts present, for the first time in this series, fibrosis and hyaline change, but this is infrequent and slight Note the cystic collecting tubule of the outer part of the medulla Magnification, 150

In the forty-six and sixty-one day specimens and especially in the ninety day specimen (fig 32) cystic dilatation of Bowman's space is marked in certain glomeruli. Some of these cystic glomeruli lack proximal convoluted tubules, but in no case does the cystic dilatation extend into the proximal convoluted tubules, which commonly appear as minute twigs opposite the vessel pole.

The "concentration" of glomeruli due to their approximation is evident early and is especially apparent in the dissected specimen in the later stages, when the cortex is largely composed of glomeruli separated only by connective tissue fibrils and minute unidentifiable tubular remnants.

*Proximal Convoluted Tubules*—(a) Microscopic Sections. From the first day of obstruction marked change is evident in the proximal convoluted tubules (fig 4). The lumens of some are increased in caliber, and their epithelial cells are variably flattened against the basement membranes, other tubules are compressed, and in some the epithelial cells present marked parenchymatous degeneration or considerable vacuolation of their cytoplasm. Mitotic figures are not uncommon among the epithelial cells of these elements in the three and six day specimens but are not seen later. At no time is there evidence of bleeding into the proximal convoluted tubules.

In the ten day specimen there is apparent a topographic distribution of the atrophy that will be considered later. In somewhat more advanced stages, with progressive atrophy of cortical tubules and increasing proliferation of connective tissue, there develops the admitted difficulty in the identification of the tubules in microscopic sections. However, small "groups" of markedly atrophic proximal convoluted tubules are easily identifiable after four or five weeks (fig 6).

(b) Microdissection. In the material of this study hypertrophy of the proximal convoluted tubule was not encountered nor was the external diameter of this element ever found increased over normal by dilatation. A perceptible shortening and a definite reduction in caliber are, however, evident in many of the proximal convoluted tubules after twenty-four hours' obstruction.

In the three and six day specimens the atrophy is considerably increased (figs 11, 18 and 19) and the largest proximal convoluted tubule encountered is definitely atrophic and of increased fragility. The variation in caliber and in refractive qualities is segmental in character and irregularly placed, and adjacent proximal convoluted tubules commonly vary in size and in the degree of change at any particular level. The only change found localized within individual tubules with any constancy is a lesser decrease in refractive qualities of the initial portion of the tubule and increased thinness, delicacy and translucence of the



## EXPLANATION OF FIGURES 10 TO 16

Fig 10 —Complete nephron and collecting tubules from a normal kidney Magnification, 20

Fig 11 (rabbits 20 and 21) —Atrophy of the proximal convolutions in the central area after three and six days' obstruction Note the reduction in size of the glomeruli Magnification, 20

Fig 12 (rabbit 7) —Extreme atrophy of glomeruli and proximal convolutions in the lateral area after fourteen days' obstruction Magnification, 20

Fig 13 (rabbit 15) —Proximal convolution from the intermediate area after twenty-one days' obstruction Contrast the initial and terminal portions Magnification, 20

Fig 14 (rabbit 16) —Well preserved proximal convolution from the intermediate area and the only dissectable proximals from the central area after twenty-eight days' obstruction Note similar glomeruli on both types of nephrons Magnification, 20

Fig 15 (rabbit 20) —Duct of Bellini and lower collecting tubules after three days' obstruction, showing little change Magnification, 20

Fig 16 —Duct of Bellini and lower collecting tubules from a normal kidney Magnification, 20

10

12 13

15

## EXPLANATION OF FIGURES 17 TO 22

Fig 17 (rabbit 14) —A stretched collecting tubule to the lateral area after ten days' obstruction Magnification, 20

Fig 18 (rabbit 21) —An atrophic proximal convolution from the central portion after six days' obstruction Contrast the length of this tubule with the tubule on the right in figure 11, which is from the same zone of the same kidney Magnification, 20

Fig 19 (rabbit 21) —A fairly large proximal convolution from the lateral area after six days' obstruction Note the variability from one portion to another Magnification, 20

Fig 20 (rabbit 14) —Dilatation of collecting tubules in the central area after ten days' obstruction Magnification, 20

Fig 21 (rabbit 15) —Obliteration of distal convolutions in the central area and the formation of budlike structures on the collecting tubule after twenty-one days' obstruction Magnification, 20

Fig 22 (rabbit 15) —Cystic dilatation of the connecting piece after twenty-one days' obstruction Magnification, 20



Figures 17 to 22

terminal segment (fig 13) In later stages this fragile, dilated terminal segment becomes molded by developing fibrosis, with the production of saccular deformities, and persists in some instances for several weeks after the remainder of the tubule has lost its identity as a dissectable structure (fig 24)

At ten days a topographic distribution of the atrophy within the organ becomes evident (figs 45 and 46) At the end of fourteen days the atrophy of many of the proximal convoluted tubules is marked (fig 12), and in review of specimens, including that of twenty-one days, it appears that the rate of atrophy is greatest in the first week At twenty-eight days dissectable proximal convoluted tubules are uncommon save in one certain area of the organ (figs 23 and 25), and in the thirty-six day and subsequent specimens they are too atrophic for dissection save in this same limited region (figs 28 and 29) In the ninety, one hundred and sixty-three and two hundred and thirty-one day specimens no dissectable proximal convoluted tubules are found, although a few relatively well preserved elements were present in the one hundred and fourteen day specimen (fig 28)

*Loops of Henle*—(a) Microscopic Sections In the first weeks the loops of Henle commonly present a dilatation of their lumens with flattening of the epithelial cells and often contain coagulated fluid (figs 4 and 5) Later certain of them are compressed by the dilatation of the collecting tubules between which they lie and also by the proliferating interstitial tissue Some are also observed to extend into the margin of necrosis lining the fragmented papilla

(b) Microdissection The transition of the proximal convoluted tubule to the thin segment of Henle's loop is sharp and distinct and is consistently found in all the specimens isolated (figs 11-14 and 23) The boundary between the *Zwischenstück* (middle piece) and the distal convoluted tubule is never as distinct as that between the proximal convoluted tubule and the thin limb, but the dilatation of the distal convoluted tubule to be described later never extends into the loop of Henle Although the loop of Henle becomes increasingly thin and delicate and correspondingly more difficult to isolate as an intact structure, it can be traced with sufficient accuracy to indicate that it retains its identity without any extraordinary shortening through an interval of thirty-five days, after which it is usually impossible to separate the structure with any satisfaction from the connective tissue fibrils and atrophic tubular remnants When Henle's loops were found to be compressed or even interrupted by pressure of adjacent dilated collecting tubules or by encroachment of connective tissue they were not found to be dilated proximal to the stenosis or occlusion Indeed, an increase in the external diameter of a loop of Henle was never observed at any time during the development of the lesion (figs 20, 21, 30 and 31)

*Distal Convolted Tubules*—(a) *Microscopic Sections* After twenty-four hours' obstruction there is considerable dilatation of the lumens of the distal convoluted tubules with flattening of the epithelium against the basement membranes (figs 4 and 5) Their lumens frequently contain coagulated fluid, which, however, does not appear as a cast capable of obstructing the lumen In the first weeks there is little additional change save that the epithelial atrophy is more marked and that an actual increase in external diameter occurs In the ten and twenty-one day specimens a few well preserved erythrocytes are present in the distal convoluted tubules in the central area of the organ

After twenty-one days the distal convoluted tubules are not identifiable by their histologic appearance in sections with the exception of those in one particular region of the kidney These can be recognized, in fact, only by reference to the appearances noted in dissected material, where after thirty-six days' obstruction they are found to be enlarged and filled with blood (fig 7) Blood is not observed elsewhere in the kidney save in the associated collecting tubules In all the later specimens blood is found in similar locations, with the red cells progressively fused and hyalinized and with granules of hemosiderin appearing within them

(b) *Microdissection* In the first few days of obstruction the contour of the distal convoluted tubules is unchanged, but they become increasingly more fragile and are of decreased refractive qualities At six days there is an infrequent slight increase in external diameter, and this becomes somewhat more marked at ten and fourteen days (figs 17 and 20) There may be variability in the degree of distention of the distal convoluted tubules joining one collecting tubule The increase in external diameter in no case is found to extend into the ascending limb of Henle but frequently is continuous with that of the connecting piece and collecting tubule The latter, together with the first part of the distal convoluted tubule in the twenty-one, twenty-eight and thirty-six day specimens, presents prominent local cystic dilatations highly irregular in distribution (figs 21, 22, 27 and 31) The walls of these cystic structures are exceedingly thin and delicate, and some are found to end blindly as they are dissected from the connective tissue in which they are embedded

At thirty-six days greatly increased distention and deformity of certain of the distal convoluted tubules are observed, and they are filled with blood The latter is confined almost entirely to loops of the distal convoluted tubules, being present elsewhere, in small amount, only in the cortical portions of the corresponding collecting tubules (fig 40) From forty-six days to two hundred and thirty-one days pigmented distal convoluted tubules are found in similar distribution With increasing

## EXPLANATION OF FIGURES 23 TO 33

Fig 23 (rabbit 16) —Well preserved proximal convoluted tubule from the intermediate area after twenty-eight days' obstruction Magnification, 20

Fig 24 (rabbit 16) —Terminal portion of a proximal convoluted tubule from the intermediate portion of the kidney after twenty-eight days' obstruction Note the variability in caliber Magnification, 20

Fig 25 (rabbit 17) —Increased atrophy of the proximal convolution in the intermediate area after thirty-five days' obstruction Magnification, 20

Fig 26 (rabbit 16) —One of the very few dissectable atrophic proximal convolutions from the lateral area after twenty-eight days' obstruction Magnification, 20

Fig 27 (rabbit 17) —Cystic dilatations of the distal convoluted tubules in the intermediate zone after thirty-five days' obstruction Magnification, 20

Fig 28 (rabbit 13) —Atrophic proximal convolutions from the central area after one hundred and fourteen days' obstruction Magnification, 20

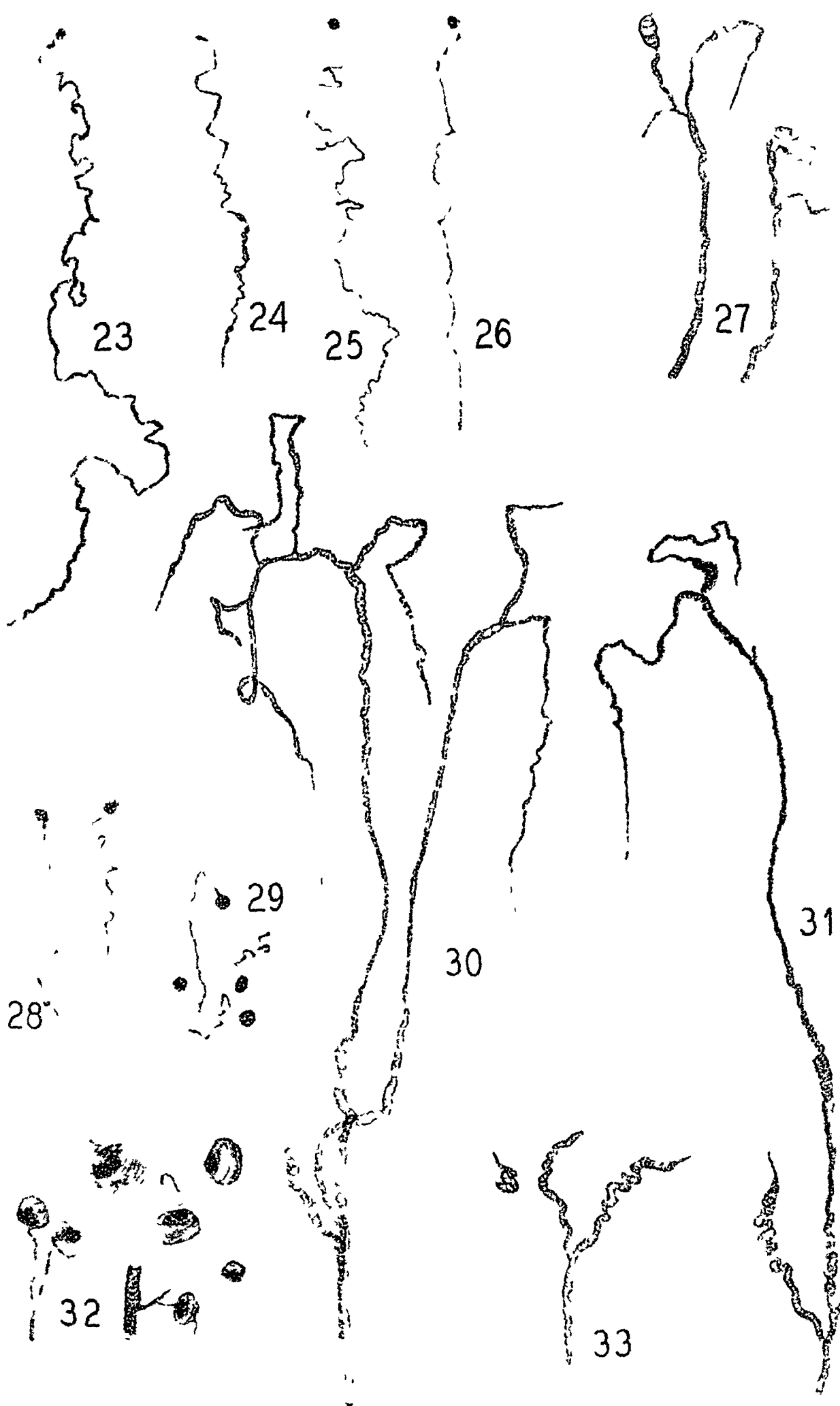
Fig 29 (rabbit 8) —Atrophic proximal convolutions from the lateral area after forty-six days' obstruction Magnification, 20

Fig 30 (rabbit 16) —Dilated and deformed central collecting tubules from the central portion after twenty-eight days' obstruction Magnification, 20

Fig 31 (rabbit 16) —Dilated, deformed and interrupted collecting tubules from the central area after twenty-eight days' obstruction Note the dilated connecting pieces Magnification, 20

Fig 32 (rabbits 9 and 19) —Dilatation of Bowman's spaces of glomeruli in the central and lateral areas after ninety days' obstruction One is from a sixty-one day kidney Magnification, 20

Fig 33 (rabbit 16) —Remnants of central collecting tubules from the inner zone of the medulla after twenty-eight days' obstruction Magnification, 20



Figures 23 to 33



## EXPLANATION OF FIGURES 34 TO 42

Fig 34 (rabbit 8) —Pigmented distal convolutions from the central area after forty-six days' obstruction Magnification, 20

Fig 35 (rabbit 19) —Remnants of pigmented distal convolutions from the central area after sixty-one days' obstruction Magnification, 20

Fig 36 (rabbit 19) —Blind and flattened collecting tubules to the lateral zone after sixty-one days' obstruction Magnification, 20

Fig 37 (rabbit 13) —Atrophic, blind and flattened collecting tubules to the lateral area after one hundred and fourteen days' obstruction Magnification, 20

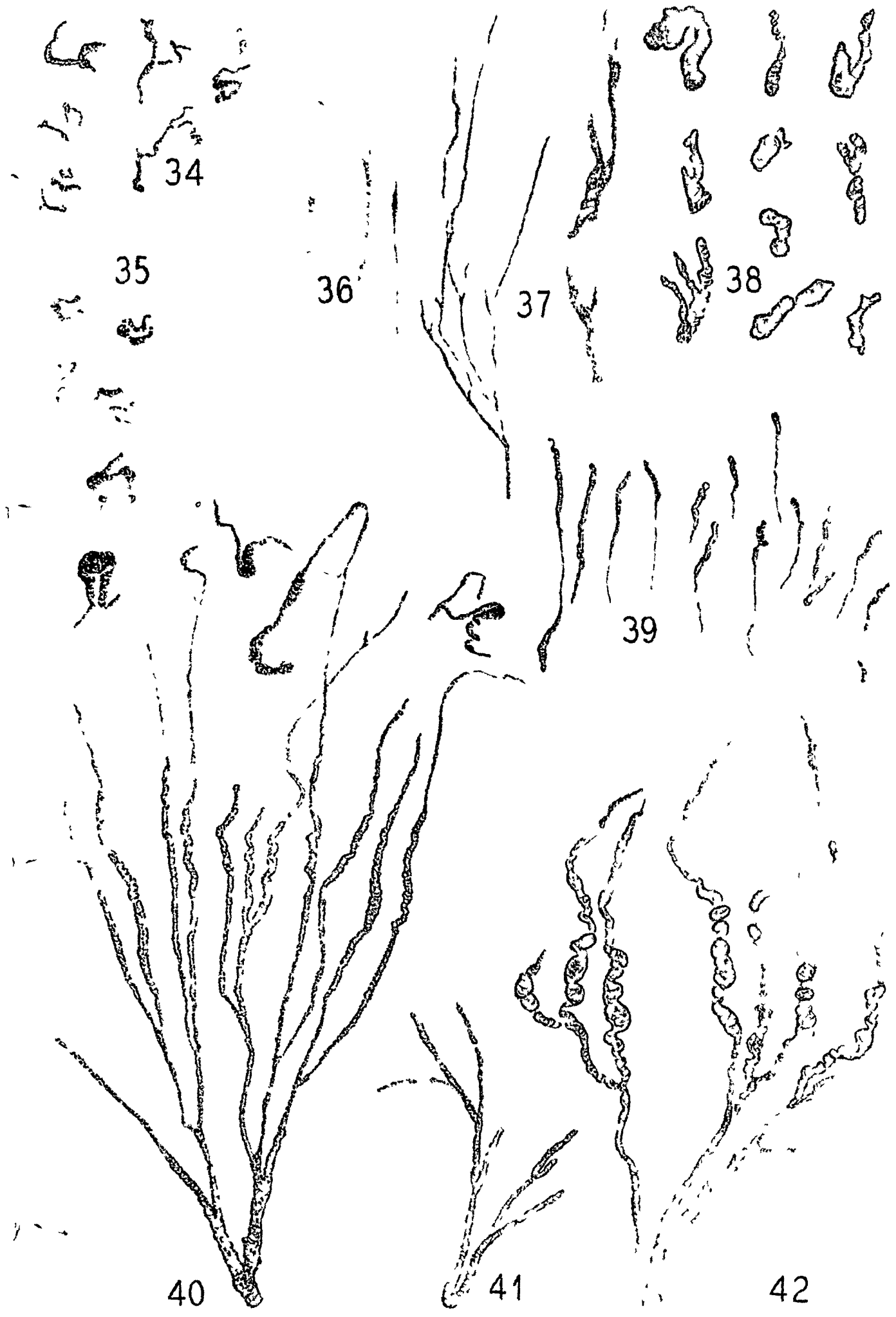
Fig 38 (rabbit 10) —Blind cystic remnants of collecting tubules from the central portion of the medulla after one hundred and sixty-four days' obstruction Magnification, 20

Fig 39 (rabbit 11) —Remnants of central cortical collecting tubules after two hundred and thirty-one days' obstruction Magnification, 20

Fig 40 (rabbit 17) —Collecting tubules and pigmented distal convolutions from the central portion after thirty-five days' obstruction Magnification, 20

Fig 41 (rabbit 11) —Remnants of branching medullary collecting tubules from the intermediate area after two hundred and thirty-one days' obstruction Magnification, 20

Fig 42 (rabbit 19) —Dilated collecting tubules from the central area after sixty-one days' obstruction Note the tapering blind ends at the corticomedullary border Magnification, 20



Figures 34 to 42

frequency they are cut off from both their collecting tubules and Henle's loops and so lie free, decreased greatly in size and identifiable only by their characteristic contour and pigment content (figs 34 and 35) In the lateral portion of the organ dissectable distal convoluted tubules are not evident after a few weeks, but in the intermediate area one can find distal convoluted tubules of nearly normal contour in association with better preserved proximal convoluted tubules to one hundred and fourteen days

*Collecting Tubules*—(a) *Microscopic Sections* After twenty-four hours' obstruction considerable and widespread dilatation of the lumens and compression of the epithelial cells of the collecting tubules are evident This is increased in the three and six day specimens and generally is more marked in the cortical collecting tubules (fig 4) Many of the tubules contain coagulated fluid which, as in the distal convoluted tubule, does not have the appearance of casts capable of obstructing the lumen Those ducts of Bellini when seen in section prior to their previously mentioned fragmentation are lined by epithelium which is not flattened but fairly normal in appearance

After a week the fractured ends of the larger collecting tubules communicate with the pelvic cavity, and the margin of the disrupted papillary tissue is lined by necrotic material and purulent exudate In succeeding weeks there follows increasing proliferation of connective tissue about the terminal portions of the collecting tubules, and in the twenty-eight day specimen they are completely surrounded by connective tissue, and the continuity of the pelvic epithelium is reestablished by regeneration of flattened epithelial cells of indifferent character In the sixty-one day and subsequent specimens the collecting tubules are separated from the pelvis by this proliferating connective tissue, which is covered by an uninterrupted epithelial layer

At ten days and later there is evident a topographic distribution of change in the collecting tubules, which is seen best in the dissected material The tubules from the central portions of the cortex are increasingly dilated and shortened, while those from the periphery are stretched and narrowed At fourteen days there is evident narrowing of the collecting tubules at the corticomedullary boundary, in which region the tubules are later interrupted and when finally a dense hyaline scar appears (fig 53)

In the ten and twenty-one day specimens a few erythrocytes are found in the central collecting tubules, and in the thirty-six day specimen a slight amount of blood is found in the cortical extremities of some of the central collecting tubules

In the forty-six day specimen the tubules to the lateral area have largely disappeared, and their remnants are enclosed in connective tissue However, the capillaries between them persist and are filled with blood

In the ninety day specimen between groups of glomeruli one finds groups of cystic cortical collecting tubules that can be identified by reference to the dissected material as remnants of the medullary rays.

In the one hundred and sixty-four and the two hundred and thirty-one day specimens the cystic collecting tubules of the central portion of the medulla are enclosed in abundant connective tissue and are composed of cuboid epithelial cells with well defined cytoplasmic borders and round or oval nuclei (fig 9).

(b) *Microdissection* In the first days of obstruction the collecting tubules generally become increasingly fragile and less refractive, especially in the outer portion of the medulla and cortex, and they also present a slight increase in external diameter in the region of primary branching, in the inner zone of the medulla. The large terminal collecting tubules are at first unchanged (figs 15 and 16), but after six days, when a definite general increase in external diameter of the remaining collecting tubules is evident, they are not found save as fragments.

At ten days a well defined topographic distribution of the alteration in the collecting tubules is apparent. Those of the central area are dilated fairly uniformly from the pelvis through the connecting piece and distal convoluted tubules (fig 20). At fourteen days their caliber is decreased at the corticomedullary border, and in the medulla they present an irregular series of minute indentations due to encroachment of connective tissue. In later stages this is increased until they present definite saccular deformities and pursue a tortuous course through the connective tissue enclosing them.

At twenty-eight days, in the inner portion of the medulla some tubules are found completely interrupted, with the blind ends lying adjacent in the softened collagen of the macerated tissue (figs 31 and 33). At thirty-six days the central collecting tubules are narrowed in the region of primary branching and in the cortex, with associated greater dilatation and irregularity of outline in the outer zone of the medulla (fig 40). At forty-six days the number of interrupted central collecting tubules is considerably increased. At sixty-one days the central collecting tubules end blindly at the pelvis and also at the corticomedullary border, but the saccular deformity of the persisting portion in the outer zone of the medulla is increased (fig 42). The cortical collecting tubules frequently are interrupted, forming fusiform structures, which occasionally show cut-off stubs of their branches.

In the remaining specimens of ninety, one hundred and fourteen, one hundred and sixty-four and two hundred and thirty-one days the central collecting tubules persist as blind structures of varied size and form. At ninety days those of the medullary rays are represented by linear series of minute spherical cysts. At two hundred and thirty-one days a few

minute remnants of the cortical collecting tubules persist, some reach the cortical surface and there branching, are connected by narrow epithelial remnants to minute blind pigmented distal convoluted tubules (fig 39) In the central portion of the medulla blind spherical, cylindrical or branching structures are enclosed in the abundant connective tissue (fig 38) The centrally placed tubules are more rounded, while laterally the tubular remnants become increasingly slender, flattened or fusiform (fig 41)

In contrast, the collecting tubules to the lateral portion of the organ are found at ten days to be greatly stretched and thinned and some virtually obliterated in their midportions (fig 17) In succeeding weeks increasing numbers of more medially placed collecting tubules become similarly involved At thirty-six days, in the connective tissue adjacent to the pelvic mucosa are found innumerable minute blind fusiform remnants of these collecting tubules With advance of the lesion these become more frequently interrupted, smaller, more abundant and flattened, and finally identifiable only by branches in the inner portion of the medulla or cortex (figs 36 and 37)

The intermediately placed collecting tubules are slightly and uniformly dilated at ten days In appearance they vary with the distance from the central area, on the one side resembling the deformed shortened central tubules and on the other the more slender fusiform structures of the lateral area In the midportion of this intermediate area they persist fairly straight and uniform in caliber to one hundred and fourteen days and are not interrupted in their course, being in continuity with their distal convoluted tubules and loops of Henle The possibility that some of these well preserved collecting tubules in the one hundred and fourteen day specimen communicate with the pelvis is not satisfactorily excluded, and in any event it seems that they must have done so shortly before the end of this period

#### INTERSTITIAL ALTERATIONS

In the first few weeks the interstitial tissue is edematous, but this becomes progressively less conspicuous after the third week

The interstitial tissue is promptly infiltrated by polymorphonuclear leukocytes and round cells, and these are constantly present in the hydronephrotic kidney and, although varying in degree in different specimens, become more prominent with development of the lesion (figs 4 to 9) Distinct aggregates of round cells are present in the cortex as early as twenty-one days after ureteral ligation No anywise comparable leukocytic infiltration is ever observed in microscopic sections prepared in the routine manner from the opposite kidney

An increase in the size of the nuclei of connective tissue cells is generally evident after one day, and after a week a diffuse proliferation

of connective tissue is found. In succeeding weeks this becomes more pronounced with particular localizations. It is prominent about the fragmented remnants of the collecting tubules of the papilla and also in the medulla about the collecting tubules of the central area. Here at ten days compression of the loops of Henle and serial constriction of the collecting tubules are evident, with later production of saccular deformities, linear series of spherical cysts and the bizarre structures of the advanced stages (fig. 38).

Discrete scars are evident early about the larger blood vessels. At fourteen days the central collecting tubules are narrowed at the cortico-medullary boundary, at sixty-one days they are interrupted at this point, and later in this region a dense zone of hyalinized collagen is present (fig. 53).

In this study both in the microscopic sections and in the dissected material structural change in the blood vessels appears insignificant. In the early stages there is considerable congestion of the capillaries and veins without topographic localization in the sections examined. The glomerular capillaries are quite uniformly compressed, and their blood content is less than normal.

After ninety days moderate thickening and hyalinization of the smaller arterial branches are observed. Connective tissue proliferation within the capillary tuft is seen first after two hundred and thirty-one days.

The thickening of the arterioles in the cortex (fig. 9) may be due in part to the concentration of glomeruli with associated shortening of the vascular tree.

#### TOPOGRAPHY OF HYDRONEPHROTIC KIDNEYS

For purposes of illustrating the topographic relations of the altered nephrons and collecting tubules the experiments have been grouped according to their possession of certain common attributes. The outline of a renal section typical of each period has been traced, and camera lucida drawings of actually dissected structures placed within it in the positions noted at the time of dissection. Thus the topographic figures 43 to 54 are not diagrammatic except in the sense that they represent a synthesis of the material from several kidneys.

*One and Three Days of Obstruction* (figs. 43 and 44).—In this period occur the flattening and splitting of the papilla (fig. 1) which precede separation of the fragmented ducts of Bellini. Otherwise, in this interval there is no localization of change within the organ. However, considerable and fairly widespread dilatation of tubular lumens with compression of epithelial cells rapidly develops, and this is evidenced in the dissected material by increased fragility and translucence.

of the nephrons. In addition there is a prompt and readily apparent decrease in the size of the proximal convoluted tubules.

At the end of twenty-four hours' obstruction there is a slight increase in the external diameter of the collecting tubules in the region of the primary branching, and after three days there is a slight general increase in the external diameter of most of the collecting tubules peripheral to this point.

*Six, Ten and Fourteen Days of Obstruction* (figs 45 and 46) — In this period there occurs only a slight increase in the volume of pelvic fluid, but with its accumulation there develops obliteration of the papilla with separation of the disrupted extremities of the collecting tubules. There also becomes apparent a topographic distribution of change in the nephrons throughout the kidney, and this structural pattern persists in later stages of the study.

The glomeruli in the lateral portion of the organ become flattened, with their long axes parallel to the capsular surface.

Atrophy of the proximal convoluted tubules increases and becomes especially marked in the central and lateral portions of the organ, with persistence of less atrophic units in the intermediate area.

The long loops of Henle, particularly those of the central area, extend into the marginal necrosis of the fragmented papilla, and other Henle's loops are found modified in accordance with structural alterations in the adjacent collecting tubules.

The central collecting tubules become increasingly dilated and translucent, and this change extends commonly to the loops of Henle. The collecting tubules to the lateral area are stretched and narrowed in their midportions, and some are interrupted. The collecting tubules of the intermediate area are dilated slightly and quite uniformly from the pelvis to their distal convoluted tubules.

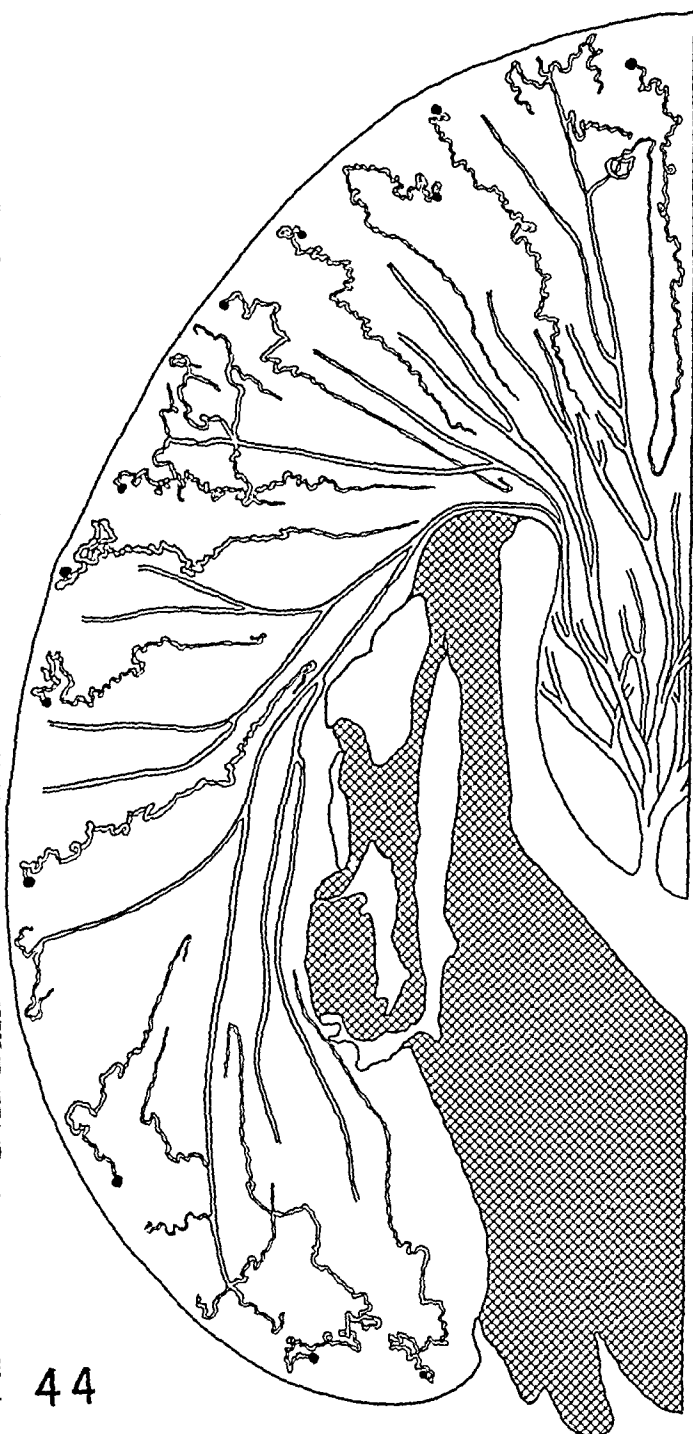
*Twenty-One and Twenty-Eight Days of Obstruction* (figs 47 and 48) — During this interval the volume of pelvic fluid is about doubled and has become blood tinged. The continuity of the pelvic and tubular epithelium is reestablished, and fibrosis within the parenchyma is conspicuous, especially in the central portion of the medulla and cortex.

Atrophy of proximal convoluted tubules is increased, and dissectable proximal convoluted tubules are rarely encountered save in the intermediate area.

The loops of Henle in the central portion of the medulla are increasingly compressed by the dilatation of the adjacent collecting tubules and by the proliferating connective tissue. Those long loops of the lateral area are stretched, and no particular shortening of the loops of Henle is apparent.



43



44

Fig 43 (rabbit 6) —One day's obstruction Magnification, 6

Fig 44—Camera lucida drawings of actually dissected structures from kidneys obstructed for intervals of one and three days placed in the position noted at the time of dissection During this interval there is no localization of change within the nephrons



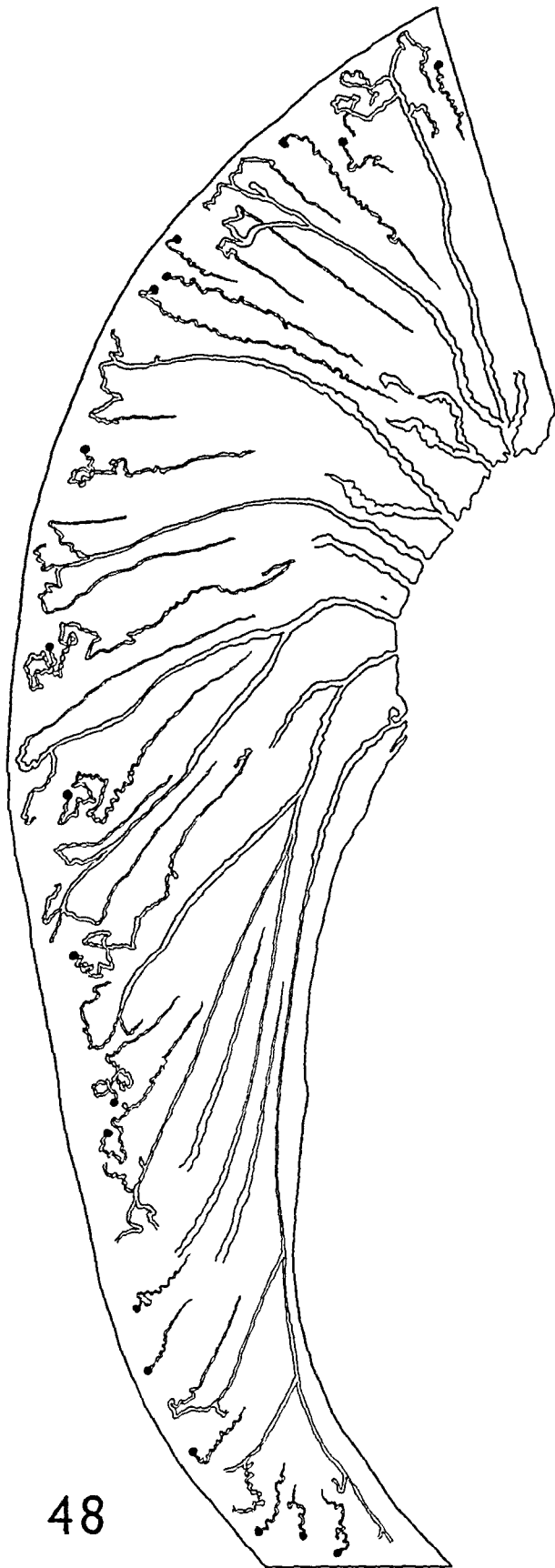


Fig 45 (rabbit 14) —Ten days' obstruction Magnification, 6

Fig 46—Six, ten and fourteen days' obstruction Topographic distribution of change is evident after ten days' obstruction This is emphasized in the increased atrophy of the proximal convoluted tubules in the central and lateral areas and in the shortening and stretching of their respective collecting tubules



47



48

Fig 47 (rabbit 16) —Twenty-eight days' obstruction Magnification, 6  
 Fig 48—Twenty-one and twenty-eight days' obstruction Few dissectable  
 proximal convoluted tubules are found save in the intermediate area

Dissectable distal convoluted tubules are very rare in the lateral area, but in the central area, although there is marked atrophy of the proximal convoluted tubules, the associated distal convoluted tubules are not reduced in volume but, on the contrary, occasionally present local cystic dilatation

The dilatation and deformity of the central collecting tubules are increased, and in the abundantly developing interstitial tissue in the inner zone of the medulla blind remnants of these tubules are observed. The collecting tubules of the intermediate area pursue a fairly straight course and present moderate uniform dilatation. In the lateral portion of the organ increasing numbers of more medially placed collecting tubules are found stretched and interrupted.

*Thirty-Six and Forty-Six Days of Obstruction* (figs 49 and 50) — In the forty-six day specimen (fig 2) the volume of the pelvic fluid is two and a half times that of the twenty-eight day specimen, and the fluid is composed in considerable part of fresh blood.

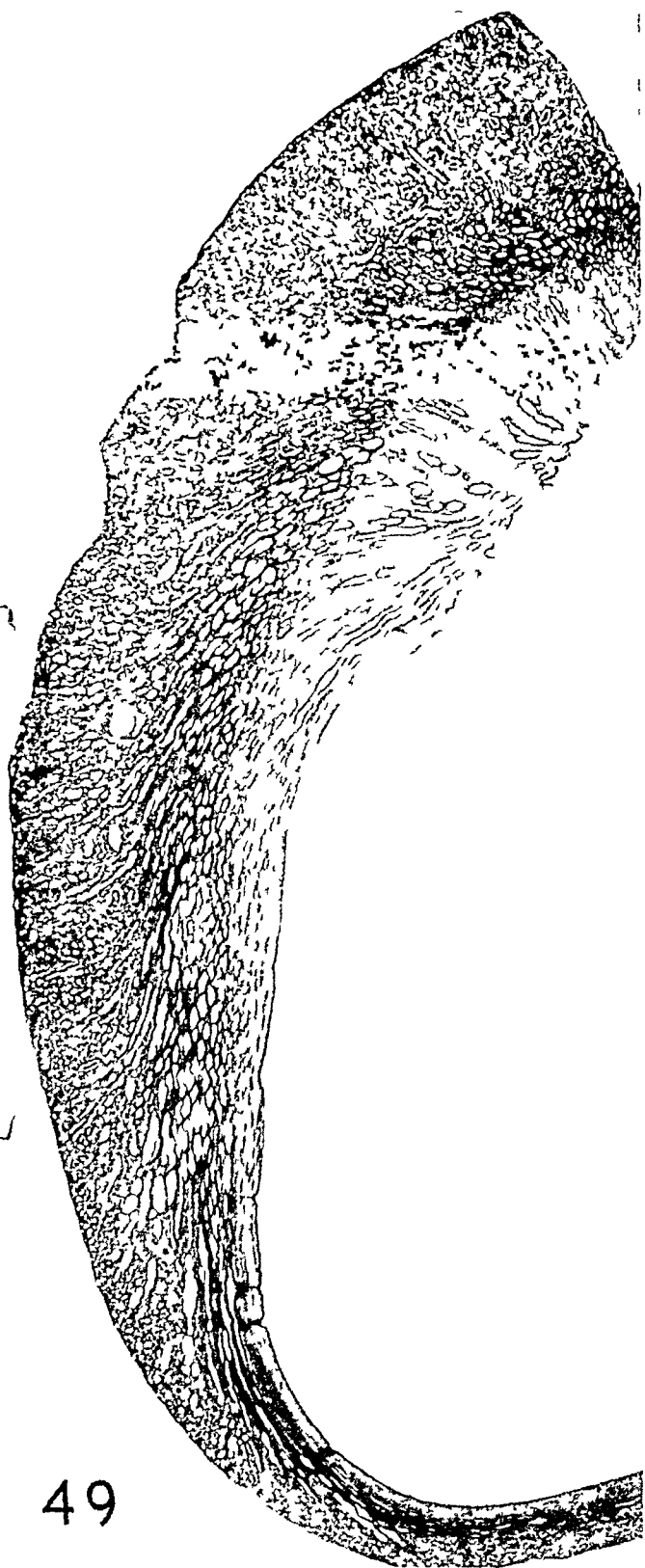
The only dissectable proximal convoluted tubules in these specimens, as in the subsequent ones, are found in the intermediate area. These are definitely atrophic, and adjacent tubules in this area vary greatly in size and in refractive qualities.

The topographic distribution of change is further emphasized in this period by an accumulation of blood in the distal convoluted tubules and to a slight degree in the collecting tubules of the central area. The lateral border of the area of pigmented distal convoluted tubules is sharp, but among better preserved elements of the intermediate area a few pigmented distal convoluted tubules are found. Several groups of these isolated distal convoluted tubules are traced to single collecting tubules, which also contain a slight amount of blood. The associated proximal convoluted tubules, identified by their relation to glomeruli, are markedly atrophic, and they, together with their glomeruli, are free from blood.

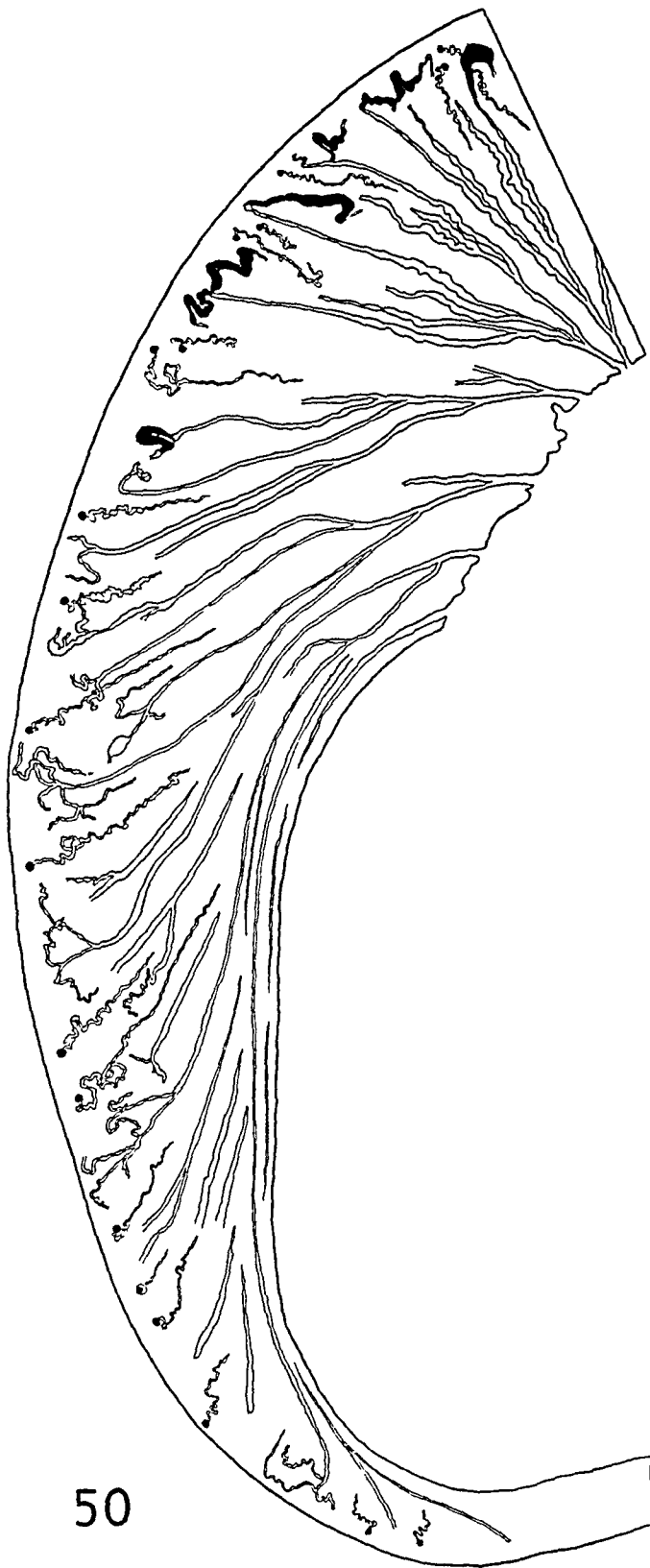
The atrophy of the collecting tubules to the lateral area has increased, and a larger proportion of these tubules is interrupted. The central collecting tubules are narrowed in the region of primary branching, are increasingly dilated and constricted in the outer zone of the medulla and are sharply narrowed at the corticomedullary boundary and through the cortex.

The less atrophic proximal convoluted tubules in the intermediate area are associated with well preserved, nonpigmented distal convoluted tubules and with collecting tubules which, although slightly dilated, pursue a fairly straight, uninterrupted course.

*Sixty-One, Ninety and One Hundred and Fourteen Days of Obstruction* (figs 51 and 52) — In this interval the volume of pelvic fluid has



49



50

Fig 49 (rabbit 17) —Thirty-six days' obstruction Magnification, 6

Fig 50 —Thirty-six and forty-six days' obstruction The topographic distribution of change is emphasized by the accumulation of blood in the distal convoluted tubules of the central area

decreased to about 25 per cent of that found in the forty-six day specimen and has become less turbid and is free from obvious blood

In the central and lateral areas there is considerable cystic dilatation of some glomeruli in the inner portion of the cortex in direct relation to the larger arcuate vessels

Persistent remnants of pigmented distal convoluted tubules occur in a distribution similar to that noted previously. In this area and in the lateral area proximal convoluted tubules are identifiable occasionally as minute twigs on glomeruli opposite the vessel pole

The central collecting tubules are interrupted at the corticomedullary border and are interrupted in the cortex repeatedly with the formation of slender fusiform structures and linear series of minute spherical cysts. The collecting tubules of the lateral area exist as blind flattened structures, identifiable with certainty only in the regions of branching in the inner portion of the medulla and cortex

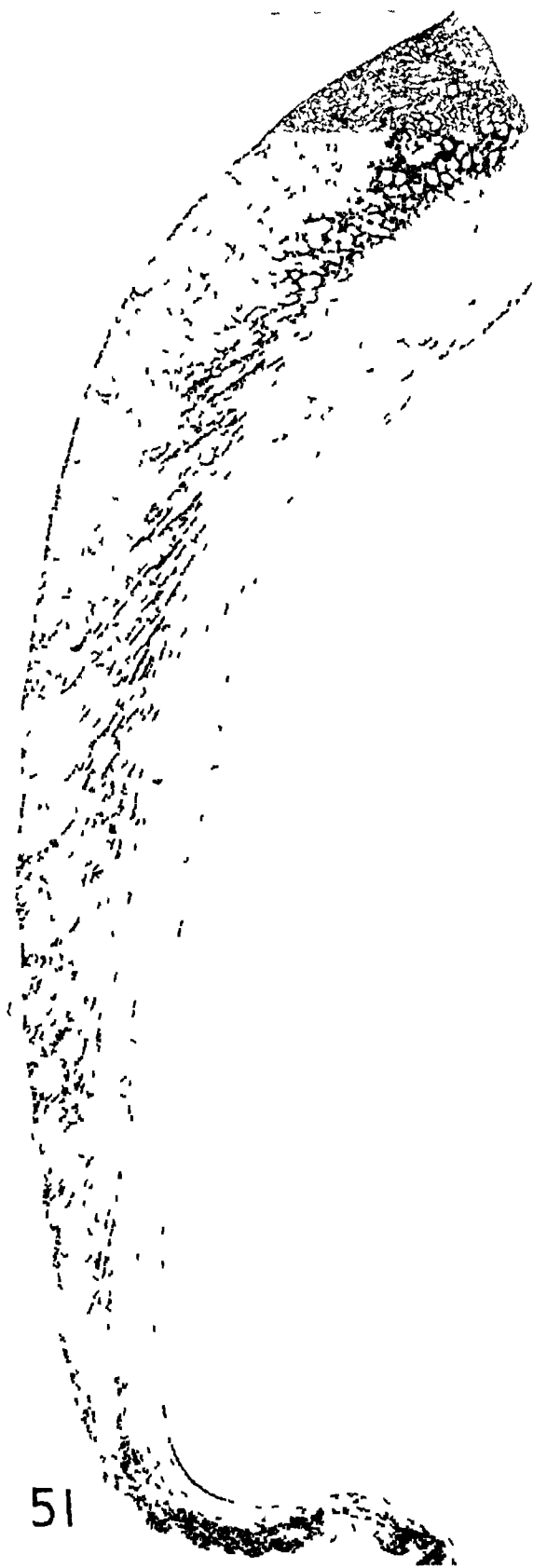
The collecting tubules are separated from the pelvis by connective tissue, and the pelvis is lined by intact epithelium

The last dissectable nephrons are found in the intermediate area of the one hundred and fourteen day specimen. Their collecting tubules, distal convoluted tubules and Henle's loops in so far as the latter could be followed, are of relatively normal contour, and their proximal convoluted tubules are slightly larger than those found in the corresponding position in the sixty-one day specimen. These elements of the one hundred and fourteen day specimen contrast with the extremely atrophic remnants of the ninety day specimen, in which no intact nephrons are found

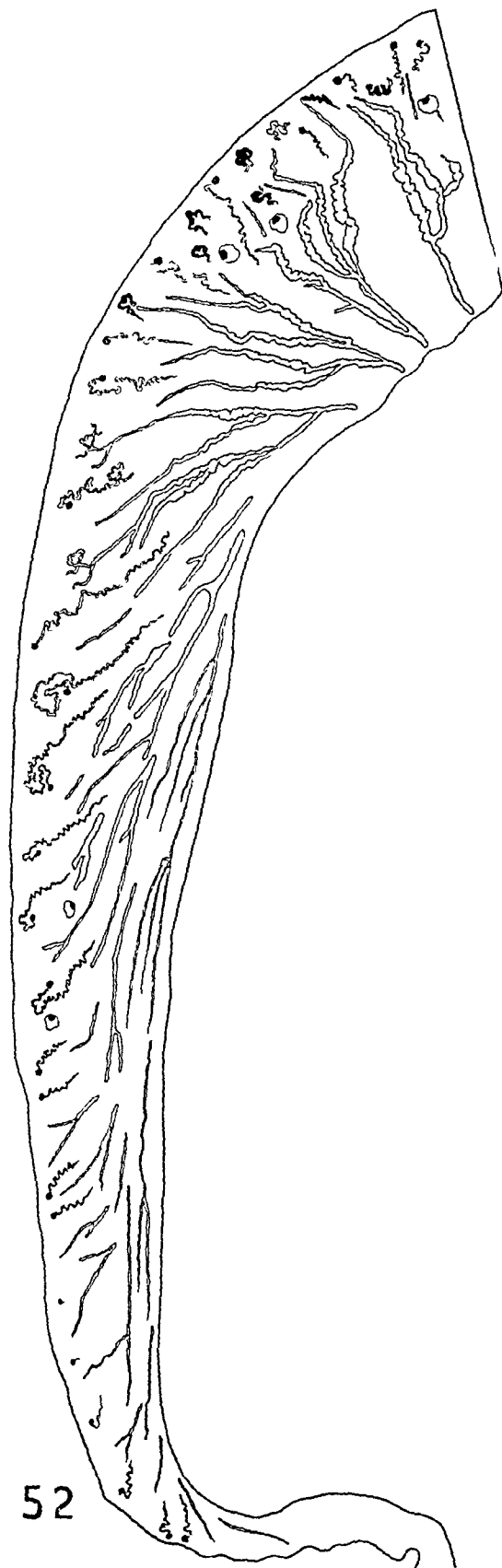
*One Hundred and Sixty-One and Two Hundred and Thirty-One Days of Obstruction* (figs 53 and 54) —During this period the volume of pelvic fluid is reduced further to 50 per cent of that at one hundred and fourteen days of obstruction, and in some dimensions the hydionephrotic kidney is reduced below the normal. The pelvic fluid however, even at this stage is not quite clear but remains slightly turbid and yellowish

The glomeruli are remarkably close together, being separated only by collagen fibrils and an occasional tubular remnant. Proximal convoluted tubules are not identifiable. Occasional blind remnants of pigmented distal convoluted tubules are found in the central portion of the cortex

The blind, cystic medullary collecting tubules are of variable size and contour, depending on their branching and their distance from the central area. With progression laterally from the central part of the medulla they become increasingly more slender and shorter until they blend with the exceedingly atrophic, undissectable remnants of the collecting tubules to the more lateral portions of the organ



51



52

Fig 51 (rabbit 19) —Sixty-one days' obstruction Magnification, 6

Fig 52—Sixty-one, ninety and one hundred and fourteen days' obstruction Cystic dilatation of the glomeruli is noted in the central and lateral areas Pigmented remnants of distal convoluted tubules persist in the central area The last dissectable nephrons are found in the intermediate area in the one hundred and fourteen day specimen

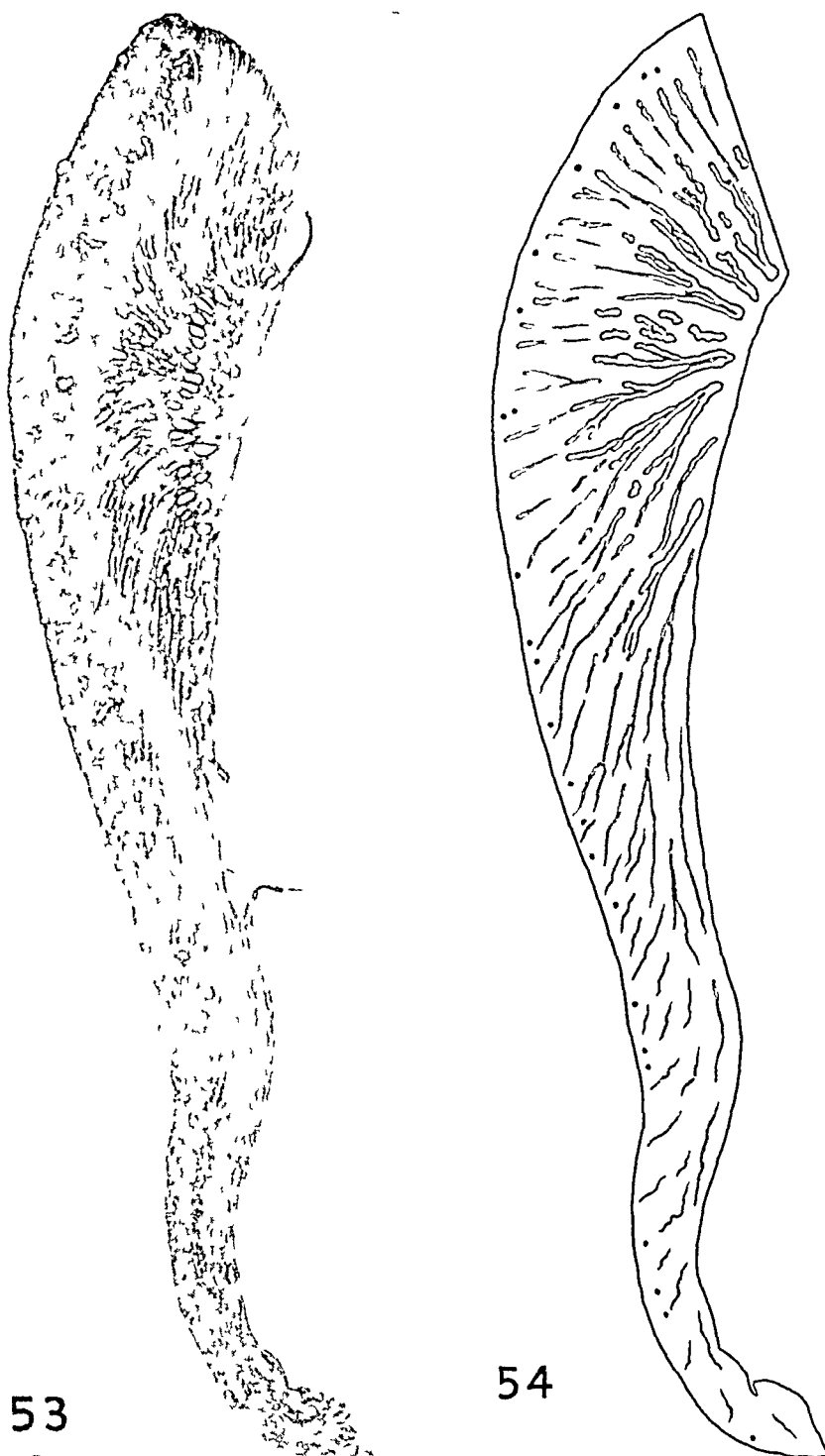


Fig 53 (rabbit 11) —Two hundred and thirty-one days' obstruction Magnification, 6

Fig 54—One hundred and sixty-one and two hundred and thirty-one days' obstruction The cortex is composed of concentrated glomeruli separated by collagen fibrils and minute unidentifiable tubular remnants The collecting tubules of the medulla vary in size and contour, in their branching and in their distance from the central area, blending laterally with undissectable tubular remnants

## COMMENT

For years the literature has contained Cohnheim's<sup>5</sup> authoritative description of typical hydronephrosis produced by sudden, permanent experimental occlusion of the ureters of rabbits and dogs. Although he contrasted the "moderate" hydronephrosis developing after sudden, persistent ureteral occlusion with the really voluminous hydronephrosis found in animals with slowly developing, partial or intermittent obstruction, his observations offer no basis for confusion and are in accord with common experience. He described the yielding of the pelvis to the accumulating fluid and the setting in of absorption of the fluid. He believed it unnecessary to have recourse to the possibility of continued secretion by the pelvic mucosa to explain the increase in volume of the pelvic fluid because he found that the kidney had by no means ceased to secrete. His experimental results were amply confirmed several decades ago by Guyon and Albarran,<sup>6</sup> Fabian,<sup>7</sup> Ponfick<sup>1</sup> and Suzuki.<sup>8</sup>

Nevertheless, suggestions persist that sudden complete ureteral obstruction is not followed by accumulation of pelvic fluid, and from this erroneous idea, and not from direct anatomic evidence, prompt parenchymal atrophy has been inferred to account for the supposed lack of accumulating fluid. The lineage of this concept of "primary atrophy" perhaps may be traced through the early work of Lindemann<sup>9</sup> and others, but, as Papin<sup>10</sup> observed, the exceedingly infrequent experimental examples of the so-called primary atrophy were generally recognized at the time as exceptional.

Misquotation of Cohnheim<sup>5</sup> has sustained this notion of "primary atrophy." Such misinterpretation is to be found in the papers of Albarran,<sup>11</sup> Lindemann<sup>9</sup> and Scott<sup>12</sup> and more recently in the work of Suter.<sup>13</sup>

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5 Cohnheim, J. *Vorlesungen über allgemeine Pathologie*, Berlin, A. Hirschwald, 1880.

6 Guyon, F., and Albarran, J. *Arch. de med. exper. et d'anat. path.* **2**: 181, 1890.

7 Fabian, E. *Pathologie und pathologische Anatomie*, in Born, G., and Flugge, K. *Bibliotheca medica*, Cassel, T. G. Fischer & Co., 1904, vol. 18, p. 1.

8 Suzuki, T. *Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen*, Jena, Gustav Fischer, 1912.

9 Lindemann, W. *Ztschr. f. klin. Med.* **34**: 299, 1898.

10 Papin, E. *Les hydronéphroses*, Paris, Gaston Doin, 1930.

11 Albarran, J. *Exposé des travaux scientifiques de J. Albarran*, Paris, Masson & Cie, 1906.

12 Scott, G. D. *Surg., Gynec. & Obst.* **15**: 296, 1912.

13 Suter, F., in von Bergmann, G., and Staehelin, R. *Handbuch der inneren Medizin*, Berlin, Julius Springer, 1931, vol. 6, pt. 2.



It is to be found also in the current edition of a leading American textbook of pathology <sup>14</sup> "Cohnheim made the statement that only partial or intermittent obstruction is followed by great distension of the pelvis, while complete obstruction results in cessation of the flow of urine and atrophy of the kidney "

Although this concept of "primary atrophy" had no acceptable demonstrated basis in reality, and although it was adequately refuted more than three decades ago, the extreme expression of a converse conception is found in Hinman's <sup>15</sup> opinion that the "pyclogenous back-flow" from the pelvis of an actively functioning hydronephrotic kidney permits maintenance of a "fresh water lake continuously renewed by active secretion above "

Ponfick's <sup>1</sup> studies led him to conclude that the rate of accumulation of pelvic fluid and the rate of atrophy of the parenchyma are in agreement, that reciprocal relations exist between the mass of the pelvic fluid and the mass of the parenchymal elements. For this reason it appeared to him that in later stages and in higher degrees of pelvic distention an ever increasing proportion of the accumulating fluid must flow from the pelvic mucosa. In the present study the rate of accumulation of pelvic fluid and the rate of atrophy of the parenchyma are found to be in accord with the corresponding data of Ponfick, and, although the pelvic content obviously is not "stagnant," it at no time resembles a "fresh water lake "

It is commonly acknowledged that the pelvic fluid quickly loses the character of urine, and it must therefore be granted that the organ has ceased to function as a kidney. Although for a considerable period after ureteral ligation the highly vascular renal parenchyma seems an obvious source of pelvic fluid, and although the analogy drawn between hydronephrosis and hydrops of the gallbladder is an ancient one, no assurance has been offered thus far as to the validity of the varying opinions as to the source of the fluid. Thus the accumulating pelvic fluid toward which, as Ponfick stressed, attention so largely has been directed by the prejudicial term "hydronephrosis" loses its immediate importance as a direct measure of the parenchymal secretory activity and structural change.

The present study has shown that the rate of accumulation of pelvic fluid rises sharply between four and eight weeks after ligation of the ureter, and this change in rate is due largely to bleeding into the pelvis. Inasmuch as no source of bleeding has been found within the parenchyma

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<sup>14</sup> MacCallum, W. G. Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1938, p. 438.

<sup>15</sup> Hinman, F., in Nelson Loose-Leaf Living Surgery, New York, Thomas Nelson & Sons, 1937, vol. 6, p. 545.

even on histologic examination, it is assumed that the hemorrhage may be from peripelvic veins. This rapidly developing hemorrhagic character and sharp increase in volume of the pelvic fluid within a limited interval of time have not heretofore been recognized. Although these features were completely reproducible in a series of 6 additional animals used for verification of these points, and although in this aspect of the problem as in others there is, in general, progressive agreement in the course of the process in the animals studied, and although in the literature there is indirect confirmation, one can conceive of circumstances which might lead to nonappearance of the bleeding, such as an unusual distribution or character of peripelvic vessels or a failure of pelvic fluid to accumulate to the critical volume required to produce the striking circulatory disturbance which in this series has constantly developed.

In the thirty-six day specimen of his series Ponfick<sup>1</sup> did not describe an especially hemorrhagic fluid but noted rust brown layers of erythrocytes on parts of the pelvic mucosa and commented on their relative abundance in the pelvic fluid in contrast to their isolated appearance in the parenchyma. He had no animals with ureters obstructed for intervals between thirty-six and one hundred and twenty days, and Fabian,<sup>7</sup> although he mentioned ligation of the ureters of 125 animals, listed none as examined in the interval between twenty-five and fifty days among the 17 for which individual protocols were offered. However, Ponfick<sup>1</sup> and Suzuki<sup>8</sup> offered undeniable corroborative evidence, shortly to be mentioned.

The early splitting of the papilla with disruption of the ducts of Bellini and later separation of the torn ends of the collecting tubules due to the accumulation of pelvic fluid is a significant injury with respect to both the regional parenchymal changes and the fate of the organ. The unipapillary kidney of the rabbit may be expected to differ somewhat from the multipapillary kidneys of other animals in this feature, and difference in the degree of trauma and in the progress of repair may be significant factors in determining the somewhat variable course this type of experimental hydronephrosis is known to follow in these animals (Fabian<sup>7</sup>). It seems likely that these factors may be responsible for the irregularities in the atrophy and in the preservation of nephrons that are so striking in the intermediate area in the ninety and one hundred and fourteen day specimens of this study.

For a variable period in the early weeks of the obstruction the surface of the fragmented papilla is necrotic and is covered by a moderately abundant purulent exudate. This "necrosis" of the papilla, described by Helmholz and Field<sup>16</sup> and assumed by them to be due to "anemia," is produced by the physical trauma that results from increas-

ing pelvic distention Kelly and Burnam<sup>17</sup> suggested that compression of the ducts of Bellini due to papillary flattening creates a valvelike structure of the papilla, and Hinman and Lee-Brown<sup>18</sup> compared this "valve" to the uretero-vesical valve and discovered that the collecting tubules become collapsed and even more "valve-like" as hydronephrosis progresses. On the contrary, as has been noted, the papilla of the rabbit kidney is torn apart by the pressure of the accumulating fluid.

Reports in the literature repeatedly speak of glomerular "dilatation" as a consequence of obstruction. This has been found to be rare and to occur, as Zurhelle<sup>19</sup> observed, only in certain glomeruli in later stages of obstruction. No significant increase in external diameter is found on measurement of glomeruli in tissue sections, and such is not evident, save as mentioned, in the macerated material, in which recognition of variation in glomerular form and volume is readily possible.

The long recognized lack of correlation between glomerular and "tubular" atrophy in hydronephrosis is evident at an early period in the dissected material. This disproportion is most obvious between glomeruli and the proximal convoluted tubules.

In the literature one encounters numerous descriptions of curious structural changes that have not been found in the present study. Among them might be mentioned the fusion between the capillary loops and the parietal layer of Bowman's capsule and the disappearance of the capsular space noted by Gruber,<sup>20</sup> the glomeruli lying loose in their capsules described by Orth<sup>21</sup> and the constant form of a flattened hexagon assumed by Bowman's capsule described by Ponfick.<sup>1</sup>

In past descriptions of the effect of hydronephrosis on the renal parenchyma there is almost universal uncertain usage of the term "dilatation." No distinction is made between increase in caliber of lumen and increase in external diameter of the tubule. The reader is therefore left uncertain as to whether any increase in the size of affected nephrons has occurred, for a tubule with a dilated lumen may or may not be a "distended" tubule.

Hypertrophy of the proximal convoluted tubules was never observed, in contrast to the finding of Hinman,<sup>15</sup> nor was the external diameter of these tubules ever found to be greater than normal, in spite of frequent mention in the literature of their "dilatation." Evidence of injury of their epithelial cells is to be found after twenty-four hours' obstruc-

17 Kelly, H. A., and Burnam, C. F. *Diseases of the Kidneys, Ureters and Bladder*, New York, D. Appleton and Company, 1914.

18 Hinman, F., and Lee-Brown, R. K. *J. A. M. A.* **82**: 607, 1924.

19 Zurhelle, E. F. *Frankfurt Ztschr. f. Path.* **10**: 42, 1912.

20 Gruber, G. B., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1934, vol. 6, pt. 2.

21 Orth, J. *Virchows Arch. f. path. Anat.* **202**: 266, 1910.

tion, and a moderate, irregularly distributed increase in caliber of their lumens is also to be seen at this time. Early degenerative change in the epithelial cells of the proximal convoluted tubules is mentioned frequently in the literature<sup>22</sup>

Suzuki,<sup>8</sup> even using intravenous injections of carmine as a means of localization and so gaining an especial appreciation of the effect of obstruction on the proximal convoluted tubules, gained only a suspicion of the rapidity and degree of their atrophy. Further, his conclusion is weakened by his frank admission that his effort to distinguish between different types of tubules in later stages of the lesion was carried to that fine point where he no longer was certain that he was telling them apart.

The most striking change in the proximal convoluted tubule is therefore their prompt and marked reduction in volume. This, indeed, is noted in the first week of obstruction but can be appreciated only in the dissected material, in which many atrophic proximal convoluted tubules can be observed at a glance and readily compared with the normal.

The most obvious condition associated with the atrophy of the proximal convoluted tubule in time and place, which usually is the essence of the establishment of a "causal relation," is urinary obstruction. The importance of this condition as the cause of parenchymal change is supported further by the more rapid atrophy occurring in the areas where secondary blockage of the tubules can be demonstrated to exist within the nephrons—i.e., in the central and lateral portions of the organ, where after twenty-eight days dissectable proximal convoluted tubules are no longer to be found—and by the fact that in the intermediate area where such secondary blockage is not to be demonstrated the least atrophic nephrons, the ones of more nearly normal contour, are to be found. Obviously, innumerable secondary causal relations develop, but thus far no one has been able to extricate himself successfully from efforts to evaluate the relative importance of, for example, the altered blood supply and interference with function and the distribution of pressure as factors in determining the genesis of hydro-nephrosis.

Thus the concept of atrophy of the kidney developing promptly after obstruction of urinary outflow appears again but established on objective anatomic evidence and including a regional distribution within the nephron and within the organ. The concept's approach to reality becomes topographic and quantitative and a function of time elapsed.

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22 (a) Heidenham, R. *Arch f d ges Physiol* **9** 10, 1874. (b) Joelson, F. J. E., Beck, C. S., and Moritz, A. R. *Arch Surg* **19** 673, 1929. (c) Boetzel, E. *Beitr z path Anat u z allg Path* **57** 294, 1913. (d) Ponfick.<sup>1</sup>

Inasmuch as the proximal convoluted tubules form the chief bulk of the organ, then rapid atrophy is a factor of the greatest importance in facilitating the accumulation of pelvic fluid and in determining the architecture of the hydronephrotic kidney.

The only localized change found with any regularity within individual proximal convoluted tubules corresponds with a localization found by Suzuki,<sup>8</sup> who noted that after nine days of obstruction, although the epithelial cells of the initial portion of the proximal convoluted tubule were almost as strongly stained by the previously injected carmine as those of the opposite side, the terminal portion of the obstructed proximal convoluted tubule was poorly stained.

Henle's loops maintain their identity through a considerable interval, being shortened or stretched only in proportion to the change occurring in the collecting tubules between which they lie embedded in connective tissue fibrils. They never become distended as do the distal convoluted tubules and collecting tubules, they are not preserved as are the glomeruli, and they show no rapid decrease in size comparable to that of the proximal convoluted tubule, from which they are continually distinguishable (as long as the proximal convoluted tubule can be dissected) by a variable but definite persistence of the original sharp transition.

The loops in the central and lateral areas become thin and delicate more rapidly than those in the intermediate area and with the increase of diffuse interstitial fibrosis are almost impossible to trace after thirty-five days. Their lumens variably increase in caliber, but no increase in external diameter is observed even proximal to obvious obstruction due to connective tissue or to dilated collecting tubules in the central region of the medulla. This interference with the loops of Henle in the central portion of the medulla, along with the tearing of the long loops involved in the disruption of the papilla, obviously provides a secondary interruption in the nephrons and involves chiefly those of the central portion of the parenchyma.

Suzuki<sup>8</sup> noted that the ascending limb of Henle is "dilated" and includes this together with the distal convoluted tubules in his dilated system of tubules. This again emphasizes the loose application of the term "dilatation." Contradictions in its use are too numerous and too unimportant to mention further save for the interesting contrast provided by Scott,<sup>12</sup> who described "dilatation" beginning in the straight tubules and proceeding through the proximal convoluted tubules to the glomeruli, and Johnson,<sup>3</sup> who described "dilatation" primarily in the glomeruli with progressive extension through the tubular system to the pelvis. In the present study the only "distended" tubules encountered have been the distal convoluted tubules and the collecting tubules of certain portions of the kidney.

In the aforementioned study by microdissection<sup>3</sup> there are described a shortening and drawing up of the long loops of Henle with the consequent disappearance of the loop so that the glomerulus comes to lie directly attached to the collecting tubule. No dissections are shown to support this conclusion, reference being made for its illustration to a diagram which represents obviously a subjective interpretation rather than a reality of observation. Such was not found in the material of the present study, but appearances that conceivably might give rise to such an interpretation were provided roughly by arteries with glomeruli attached or by collecting tubules and distal convoluted tubules with glomeruli attached. The glomeruli normally are attached to their distal convoluted tubules by fibrous strands of greater tensile strength than is possessed by the delicate neck of the proximal convoluted tubule and so may remain attached to the distal convoluted tubule on dissection of the macerated tissue.

In contrast to the absence of distention in the proximal convoluted tubules and loops of Henle at any stage of the hydronephrotic alteration, an increase in the external diameter of many of the distal convoluted tubules and collecting tubules develops early and not infrequently reaches for a time cystic proportions. In the kidneys of a patient with Bright's disease in the terminal stage Oliver<sup>23</sup> found remarkable dilatation of Henle's loops and of proximal convoluted tubules as a result of obstruction due to filling of dilated distal convoluted tubules and collecting tubules with solid debris. There exists an obvious difference in the gradual development of occlusion of the individual nephrons of the kidney in Bright's disease and the sudden interference with ureteral flow in experimental hydronephrosis with the complicating factor of pelvic distention, which doubtless tends to prevent spread of intratubular pressure throughout the kidney.

On the other hand, this difference in behavior contrasts with the striking similarity of the structural form of the dilated and deformed collecting tubules found in the central portion of the medulla in the later stages of hydronephrosis (fig 42) and the pattern of collecting tubules isolated from kidneys of patients with Bright's disease in the terminal stage by Oliver<sup>24</sup>.

The value of the method of microdissection in revealing the arrangement of nephrons into an organ is well illustrated in the hydronephrotic rabbit kidney because of the relative simplicity of the general topographic pattern that is reduplicated in the successive stages of the experimental lesion. Despite this simplicity, efforts to localize such change within the kidney by a study of microscopic sections have been largely unproduc-

23 Oliver,<sup>2d</sup> p 90, plates XVIII-XXI

24 Oliver,<sup>2d</sup> plate VI, fig 44

tive In the literature<sup>25</sup> one frequently encounters the term "group atrophy" of parenchymal elements without intimation as to the constitution of the "groups" The only possibilities suggesting themselves as explaining the use of this term is that repeated cuts across a single proximal convoluted tubule or that the previously mentioned cystic tubules of the medullary rays may have been observed

Suzuki<sup>8</sup> found that the best preserved tubules were in the "medial-sagittal" section of the kidney and that the rate of atrophy was directly related to the distance from the papilla The more rapid atrophy of the lateral portion of the kidney has been generally recognized, and Suzuki attributed it to the fact that the collecting tubules adjacent to the pelvis are more readily compressed by the direct pressure of the accumulating pelvic fluid However this may be, as microdissection shows, these collecting tubules are stretched and interrupted early, and this is the chief finding related to the more prompt atrophy of the lateral area of the kidney The importance of casts as causes of secondary obstruction within the kidney, emphasized by Ponfick,<sup>1</sup> is not borne out by an examination of the tubules in their actual continuity in microdissection Detritus was rarely seen plugging tubules and then was found in tubules already interrupted

With accumulation of pelvic fluid and advance of the parenchymal lesion there is progressive narrowing of the intermediate zone of the kidney, which undergoes minimal disturbance in general arrangement This smaller distortion together with the slighter involvement of the collecting tubules and Henle's loops in the fragmentation of the papilla and the lesser proliferation of the interstitial tissue is associated with the fact that some of the nephrons in this zone maintain their identity as morphologic units for as long as one hundred and fourteen days It is to be observed that after thirty-five days' obstruction some of the proximal convoluted tubules in this area possess mitochondria of nearly normal arrangement

The distance between the pelvis and the cortical surface in the central area is quickly reduced with the accumulation of pelvic fluid, and thus there develops a considerable difference in length of collecting tubules between the central and the intermediate zones The central collecting tubules also promptly become distended, and this distention increases in certain regions to a considerable degree Contrary to the findings of Suzuki,<sup>8</sup> the atrophy of the proximal convoluted tubules in the central area is nearly as rapid as is that in the lateral area This is associated with the greater involvement of the long Henle's loops in the fragmentation of the papilla and the compression of these loops by the promptly distended

25 (a) Hinman, F Surg, Gynec & Obst 58 356, 1934 (b) Hinman, F, and Morrison, D M Surg, Gynec & Obst 42 209, 1926 (c) Hinman<sup>15</sup> (d) Joelson<sup>22b</sup>

collecting tubules and by the early and marked proliferation of connective tissue in this area. Thus, although there is rapid atrophy of the proximal convoluted tubules in both the lateral and the central portion of the kidney, the collecting tubules in the former area are stretched and interrupted while in the latter portion of the kidney they are shortened and dilated. The pressure within the central collecting tubules is evidently sufficient to maintain them together with their distal convoluted tubules as patent tubes for a considerable period after their respective proximal convoluted tubules and Henle's loops are markedly atrophied. That for a time considerable fluid flows from the pelvis through these short, dilated central collecting tubules to be absorbed in the distal convoluted tubules will be evident from the following considerations.

After thirty-six days' obstruction the distal convoluted tubules in the central area are dilated and filled with blood. The sudden increase in size and the close packing of the erythrocytes indicate that within a short period there must have been a considerable backflow of blood and absorption of fluid from the distal convoluted tubules.

The source and peculiar distribution of this blood puzzled Ponfick,<sup>1</sup> who noted it first in his thirty-six day specimen. He sensed that it might yield important information about the genesis of hydronephrosis but, being unable to identify the tubules in which it occurred, saw only that in the progressive changes occurring in the blood cells there existed a guide to the duration of the lesion.

Suzuki<sup>8</sup> found blood with identical distribution in his forty-one day specimen and also in his later specimens and noted the development of stainable iron within it. Suzuki likewise failed to localize the blood within the distal convoluted tubules—an identification impossible in the microscopic sections that is obvious in the dissected material. Suzuki thought that the blood might come from the glomerulus but was dissatisfied with this conjecture and offered as an alternative hypothesis that it might come from local rupture of vessels into adjacent tubules. Such an idea, unsupported as it is by direct evidence, need not be maintained in face of the previously described unique distribution of this blood. Furthermore, this phenomenon is coincident with massive bleeding into the pelvis of the kidney from no identifiable parenchymal source.

Backflow is manifestly impossible in the interrupted collecting tubules of the lateral area, and while it occurs in some of the nephrons of the intermediate area which have exceedingly atrophic proximal convoluted tubules, it does not occur into those distal convoluted tubules which are associated with the least atrophic proximal convoluted tubules of this area. The relative morphologic integrity of these nephrons suggests that in many of this numerically insignificant group there may be a flow directed toward the pelvis. As an important factor in hindering



backflow into the collecting tubules of the intermediate area their greater length also must be considered. Blood was not found in collecting tubules unless it had progressed to and accumulated in the distal convoluted tubules.

The dilatation and deformity of the distal convoluted tubules and the filling of their loops by solid material are similar to the finding of Oliver in the kidneys of the patient with Bright's disease<sup>26</sup> and may be considered as additional evidence that the distal convoluted tubules are the normal sites of absorption of fluid and are points where debris may be solidified into obstructing solid masses.

No changes have been found in the vessels in tissue sections other than fairly uniform anemia of the glomerular capillaries and moderate to marked congestion of the larger veins in early stages, and no changes have been found in the dissection which permit speculation as to the efficiency of these vessels. To determine the minute volume of blood flowing through them by looking at minor changes in the artefacts of microscopic sections would seem impossible. On such a basis there has been considerable speculation concerning the obvious circulatory disturbance occurring in the hydronephrotic kidney. In perfusion experiments Ghoreyeb<sup>27</sup> found evidence of retarded flow through the obstructed kidney, initially only in the presence of the accumulated pelvic fluid, but after a week on removal of the fluid. He attributed the difference in retardation of flow to proliferative changes in the interstitial tissue.

By another approach, the injection of barium sulfate gelatin and the celluloid corrosion method, Hinman and Morrison<sup>28</sup> demonstrated an altered arrangement of the larger vessels and a decrease in number of the finer arterial branches of the hydronephrotic rabbit kidney after seventy days of complete ureteral obstruction. His conclusion that "atrophy" of the finer arterial branches had occurred and his further conclusion that parenchyma persists which is in direct relationship to the major branches of the renal artery are not borne out by the results of the present histologic study or by microdissection.

In the present study the persistent parenchyma was found to be very definitely related topographically to those distortions of the organ which permitted maintenance of the integrity of its nephrons exclusive of any possible effect of blood supply, and further this area of preserved nephrons exists in the material studied in a zone at right angles to the course of the larger renal vessels.

That the examination of the vessels of this material was not neglected may be indicated by the fact that they were studied with sufficient intensity to support with objective evidence a new general concept of the organization of the media of the distributing arteries<sup>28</sup>.

26 Oliver,<sup>2d</sup> plate V, figs 35 and 37 and text figures 33 and 53

27 Ghoreyeb, A. A. J. Exper. Med. **20** 191, 1914

28 Strong, K. C. Anat. Rec. **72** 151, 1938

The edema occurring in the early weeks of obstruction was described and discussed by Ponfick<sup>1</sup> and others. Infiltration of the obstructed kidney by polymorphonuclear leukocytes and round cells is prompt, marked and persistent, and, contrary to the experience of Johnson,<sup>29</sup> no spontaneous nephritis was encountered in the right kidneys in any of the experiments of this study.

The early extensive acute inflammation and marked fibroblastic proliferation in the peripelvic tissue have been especially described by Helmholtz and Field.<sup>16</sup>

Changes in the interstitial tissue as well as epithelial injury are manifest after twenty-four hours' obstruction, and the degradation of the parenchymal elements and the connective tissue proliferation are apparently simultaneous and interdependent. Thus, unfortunately, no light is shed on the classic dispute between Ziegler and Weigert, but it is evident that the inflammatory reaction leads to fibrosis and distortion of the parenchyma in the development of hydronephrosis as it does in other forms of chronic renal disease. The ability of these parenchymal elements to resist molding and loss of continuity due to the developing inflammatory reaction, if opportunity for function is provided, is demonstrated by the experiments of Boetzel,<sup>22c</sup> Rautenberg,<sup>30</sup> Kawasoye,<sup>31</sup> Johnson<sup>29</sup> and Joelson, Beck and Moitz.<sup>22b</sup> They showed that after several weeks of complete obstruction the previously hydronephrotic kidney when offered a chance for physiologic activity is capable of an amazing anatomic restitution and when given the stimulus can become capable of carrying the total load of renal function.

Prof. Jean R. Oliver gave help and advice during the progress of this work. Elizabeth Dunn prepared the microscopic sections, Elizabeth Cuzzoitt, the drawings of dissected specimens (figs. 10 to 42), Muriel MacDowell, the illustrations of the topographic relations, and Steven Montes, the photographs.

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29 Johnson, R. A. *J. Exper. Med.* **28** 193, 1918.

30 Rautenberg, E. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **16** 431, 1906.

31 Kawasoye, M. *Ztschr. f. gynak. Urol.* **3** 172, 1911-1912.

# Case Reports

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## RETICULOENDOTHELIOSIS WITH LIPOID STORAGE

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The remarkable activity of the reticuloendothelial system in disease results in a large variety of anatomic changes. These disorders are usually classified as inflammatory hyperplasia, neoplasia or abnormal lipid storage. Gaucher's disease, Hand-Schüller-Christian disease and Niemann-Pick disease were formerly considered disturbances of lipid metabolism but are now generally regarded as primary diseases of the reticuloendothelial system. For a discussion of these diseases, the reader is referred to the papers of Foot and Olcott,<sup>1</sup> Ritchie and Meyer,<sup>2</sup> Chevrel<sup>3</sup> and Epstein.<sup>4</sup> We are indebted principally to Epstein<sup>4</sup> for the identification of the lipoids in these diseases. In Gaucher's disease one finds the storage of a cerebroside, kerafin, in the spleen, bone marrow and lymph nodes. In Hand-Schüller-Christian disease the xanthomas throughout the body contain cholesterol. In Niemann-Pick disease there is widespread storage of phospholipid.

This report concerns a case of generalized hyperplasia of the reticuloendothelial system associated with storage of a lipid, cholesterol. It presents certain clinical and pathologic features that are not typical of either reticuloendotheliosis or Hand-Schüller-Christian disease. Dr E. T. Bell, of the University of Minnesota assisted in the preparation of the report.

### REPORT OF A CASE

A 54 year old white man was well until the spring of 1938, when he noticed a gradual onset of weakness and loss of weight. In April 1938 bronchopneumonia developed, and the patient was admitted to Mount Washington Sanatorium. There was no clinical evidence of pulmonary tuberculosis, and the sputum was negative for acid-fast bacilli. The red cell count at this time was 1,200,000, with hemoglobin 25 per cent (Tallqvist). The patient recovered from the respiratory infection, but the severe anemia persisted in spite of intensive liver and iron therapy. During the summer of 1938, after the extraction of a tooth, he bled profusely. He was admitted to Luther Hospital on September 16 because of severe anemia. On admission his temperature was 98.4 F. He was undernourished and very pale. The general physical examination gave negative results except for great enlargement of the spleen. His red cell count was 1,520,000 and the hemoglobin content was 42 Gm (Sahli). The color index was 0.91. The total leukocyte count

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From the Luther Hospital, Midefart Clinic and Mount Washington Sanatorium

1 Foot, N. C., and Olcott, C. T. *Am J Path* **10** 81, 1934

2 Ritchie, G., and Meyer, O. O. *Arch Path* **22** 729, 1936

3 Chevrel, F. *Ann d'anat path* **14** 297, 1927

4 Epstein, E. *Ergebn d allg Path u path Anat* **33** 280, 1937

was 1,100 A differential count could not be made, as the smear showed only an occasional mature polymorphonuclear neutrophil These cells showed toxic changes No immaturity was noted The red cells showed poikilocytosis There was no evidence of regeneration of red cells The urine showed no abnormal features The Kahn flocculation test was negative The patient was given two blood transfusions together with iron and liver extract but showed no improvement A red cell count on September 20 was 1,320,000, with 37 Gm of hemoglobin per hundred cubic centimeters of blood His white cell count was 400 He gradually grew weaker, bronchopneumonia developed, and he died on September 21 The clinical impression was that of aplastic anemia of undetermined nature The possibility of aleukemic leukemia was considered because of the splenomegaly

*Autopsy*—Postmortem examination was done by one of us (A J Hertzog) one hour after death

The body was 185 cm in length and was well developed but emaciated, it was estimated to weigh 130 pounds (59 Kg) There were petechiae in the skin over the abdomen and anterior part of the chest The forearms showed venipuncture wounds Rigor was absent There was slight posterior hypostasis There was grade 3 edema of both feet and ankles There was no cyanosis or jaundice The pupils were equal and measured 6 mm

The peritoneal cavity contained 250 cc of straw-colored fluid The subcutaneous fat had a lemon yellow color The peritoneal surfaces were smooth and glistening The appendix was retrocecal and measured 7 cm in length The liver was large and extended 5 cm below the costal edge The spleen was greatly enlarged The diaphragm arched to the fifth rib on the right and to the fifth interspace on the left

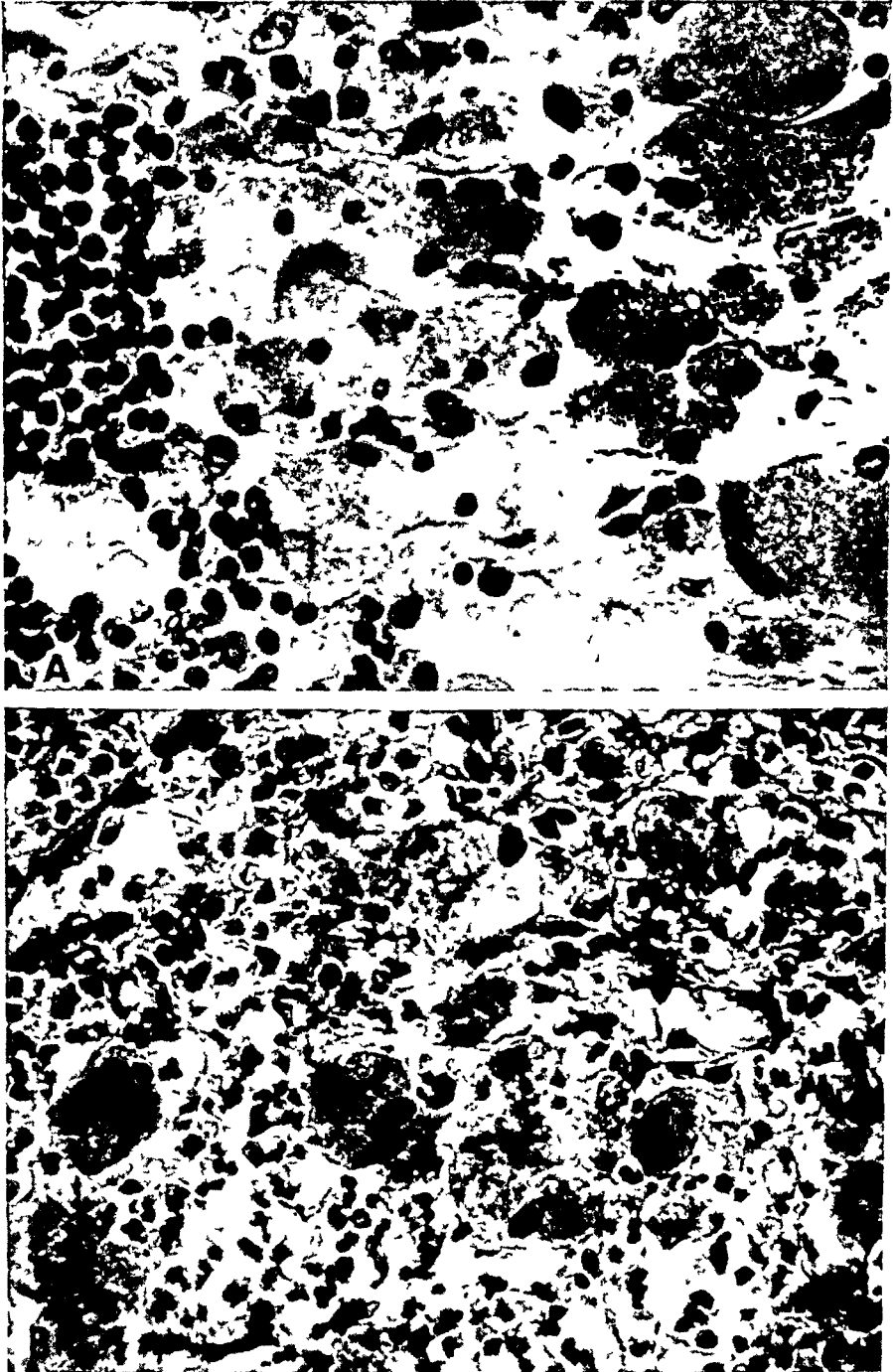
The right pleural cavity contained 1,000 cc of cloudy brown fluid There were fresh adhesions between the lateral surface of the lower lobe of the right lung and the wall of the chest The left pleural cavity contained no fluid or adhesions The pericardial sac contained a few cubic centimeters of straw-colored fluid The walls appeared normal The pulmonary artery showed no emboli

The heart weighed 400 Gm It was pale brown and had a peculiar rounded appearance The musculature was very flabby The epicardial surfaces appeared normal The auricular appendages, endocardium and valves appeared normal The septum was pale brown and showed no streaking The root of the aorta appeared normal The coronary arteries showed a minimum of sclerosis

The right lung weighed 700 Gm It was purplish gray The consistence of the lower lobe was increased The pleural surface was covered by fresh fibrinous adhesions On section the lung was dark red In the lower lobe of the right lung were nodular areas of bronchopneumonia The remaining portions of the lung showed nothing of note except edema There was no evidence of tuberculosis The right hilar nodes were greatly enlarged, grayish black and rather soft The left lung weighed 650 Gm and was mottled gray The consistence was somewhat increased, owing to partial atelectasis On section it was pinkish gray and showed slight edema There was no evidence of tuberculosis The left hilar nodes were not enlarged

The spleen weighed 1,100 Gm It was purplish blue There was a small cortical infarct, measuring 3 cm in diameter The capsule was tense, and on section the spleen was dark red The pulp was markedly congested The consistence was increased

The liver weighed 3,400 Gm. It was pale brown. On section it was pale brown and showed no mottling. The markings were distinct. The gallbladder and bile ducts appeared normal.



*A*, photomicrograph of a lymph node showing large cells filled with lipid  
*B*, photomicrograph of spleen showing clusters of large clear reticular cells

The esophagus appeared normal. The stomach contained a few cubic centimeters of brownish fluid. The walls appeared normal. The small bowel, colon and rectum showed nothing of note. The pancreas and adrenals appeared normal.

The right kidney weighed 150 Gm and the left 175 Gm. The capsules stripped easily, revealing pale brown smooth surfaces. On section the cortex and medulla were of normal thickness. The pelves and ureters appeared normal. The bladder contained some straw-colored urine.

The prostate showed grade 1 hypertrophy. The testes appeared normal. The aorta showed grade 1 atherosclerosis.

The organs of the neck appeared normal. The mesenteric lymph nodes were greatly enlarged and on section had a pale, soft appearance.

The bone marrow of the shaft of the femur was red and hyperplastic. The bone marrow of the ribs was red.

The brain and spinal cord were not examined.

*Microscopic Observations*—(a) *Lymph Nodes* The normal architecture was entirely obliterated. There was hyperplasia of the reticulum in the form of large pale foamy cells (*A* in figure). When frozen sections were stained with sudan III, the large cells were found to be filled with droplets of fat of an orange red color. The fat droplets were stained blue with Nile blue sulfate and were therefore interpreted as lipid. Some portions of the nodes showed necrosis with many polymorphonuclear neutrophils.

(b) *Spleen* The splenic pulp was markedly congested. The sinusoids were dilated. The follicles were small and indistinct. Throughout the pulp there was hyperplasia of the reticulum in the form of clusters of large clear foamy cells (*B* in figure). The fat droplets in these cells stained easily with sudan III and Nile blue sulfate.

(c) *Liver* The liver cord cells contained many large fat droplets as seen in extensive fatty metamorphosis. In addition, near the portal areas were a number of large foam cells similar to those seen in the spleen and lymph nodes.

(d) *Lungs* The alveoli contained a large amount of serum and fibrin, together with large clumps of bacteria. There was complete absence of cellular exudate.

(e) *Kidneys* The glomeruli, tubules and blood vessels showed no abnormalities.

(f) *Thyroid and Adrenals* The sections showed nothing of note.

(g) *Bone Marrow from Ribs and Femur* Sections showed hypoplastic marrow with a few clumps of large foam cells. Imprints stained with Wright and Giemsa stains showed, likewise, hypoplastic marrow with a deficiency of both red cells and granulocytes. A number of normoblasts, leukoblasts and myelocytes were seen. There were no megaloblasts. No foam cells could be seen on the imprints.

*Chemical Analysis*—Portions of the lymph nodes and spleen were ground into dry powder. An alcohol-ether extract was obtained from each. This was tested for cholesterol according to the method of Bloor. The cholesterol content of the lymph nodes was 1.71 Gm and of the spleen 1.95 Gm per hundred grams. Epstein<sup>5</sup> reported the normal cholesterol content of the spleen to be 0.62 Gm per hundred grams of dry powder.

#### COMMENT

The condition described clearly belongs to that group of reticulo-endothelial disturbances which is associated with pathologic storage of lipid. In clinical course and histologic features it resembles reticulo-endotheliosis, but in the widespread storage of cholesterol in foam cells it bears a striking resemblance to Hand-Schüller-Christian disease. Inasmuch as the latter disease is now regarded as a disease of the

reticuloendothelial system, the disease described may be interpreted as a transition between Hand-Schuller-Christian disease and malignant lymphoblastoma of the reticuloendothelial type. It may also be interpreted as an atypical form of Hand-Schuller-Christian disease.

It is to be expected that occasionally one will encounter a condition that presents characteristics common to more than one of the recognized syndromes. Epstein<sup>5</sup> described a case in which, in addition to multiple xanthomas, free cholesterol was scattered throughout the brain. Chevrel<sup>3</sup> reported a case in which properties were presented which were common to both Gaucher and Niemann-Pick disease.

#### SUMMARY

The case is reported of a 54 year old man in whom fatal aplastic anemia developed in association with marked splenomegaly. At autopsy there was widespread storage of a lipid within the cells of the reticuloendothelial system, especially of the lymph nodes and spleen. This lipid proved to be cholesterol.

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5 Epstein, E. *Virchows Arch f path Anat* 298:430, 1937.

# RUPTURED DIVERTICULUM OF THE STOMACH IN A NEWBORN INFANT, ASSOCIATED WITH CONGENITAL MEMBRANE OCCLUDING THE DUODENUM

HENRY BRODY, M D , NEW YORK

A unique combination of anomalies found in a 6 day old infant seems to be of sufficient interest to warrant reporting

Martin<sup>1</sup> collected reports of 125 cases of pulsion diverticulum of the stomach, occurring from embryonic life to senescence. Since then a number of other reports have appeared<sup>2</sup>. No case of rupture of a diverticulum has been reported.

Kellogg and Collins<sup>3</sup> collected reports of 96 cases of congenital duodenal obstruction from the literature to 1933. A number of cases have since been reported<sup>4</sup>. Fourteen cases are similar to the one reported here, the obstruction being due to a membrane, complete or with fenestration, situated in the region of the entrance of the common bile duct.

## REPORT OF A CASE

A normal-appearing newborn boy weighing 6 pounds 1½ ounces (2,764 Gm) was placed on a formula supplementing breast feeding. He took feedings fairly well but regurgitated often during the first four days of life. He passed meconium, followed on the third day by dark brown stool. On the fourth day the infant became jaundiced. On the fifth day he vomited light brown fluid containing blood clot. His temperature was 101 F. The vomiting of blood-tinged fluid continued the following day. His blood showed 5,700,000 red blood cells and 5,600 white blood cells per cubic millimeter. The hemoglobin was 144 per cent. The differential count was normal, with no abnormal cells. The platelets numbered 347,000. The bleeding and the clotting time were within normal limits. In spite of the normal blood findings, and largely because of a history of "bleeders" in the family, a diagnosis of hemorrhagic disease of the newborn was made. A transfusion of 80 cc was given. The infant continued to vomit blood-stained fluid, the temperature rapidly mounted to 105 F, and death occurred during the sixth day of life.

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From the Department of Pathology, Beth Israel Hospital

1 Martin, L. *Ann Int Med* **10** 447, 1936

2 Hess, J H, and Saphir, O. *J Pediat* **6** 1, 1935. Rivers, A B, Stevens, G A, and Kirklin, B R. *Surg, Gynec & Obst* **60** 106, 1935. Cheney, G, and Newell, R R. *Am J Digest Dis & Nutrition* **3** 920, 1936. Brown, P W, and Priestley, J T. *Proc Staff Meet, Mayo Clin* **13** 270, 1938.

3 Kellogg, E L, and Collins, J T. *Am J Surg* **30** 369, 1935.

4 Ladd, W E. *J A M A* **101** 1453, 1934. Walker, T D, Jr, Falkener, W W, and Horsley, J S. *Virginia M Monthly* **62** 141, 1935. Bonar, T G D. *Lancet* **2** 822, 1935. Regnier, E A. *Minnesota Med* **18** 60, 1935. Greenblatt, A. *Brit M J* **2** 840, 1935. Cesaris Demel, V. *Pathologica* **27** 533, 1935. Niosi, G S. *ibid* **28** 414, 1936.



*Autopsy*—Only the pertinent anatomic findings will be described. The abdomen was markedly distended. When the peritoneum was opened, a considerable amount of air under pressure escaped. The abdominal cavity contained about 200 cc of partly bloody, partly brownish-black fluid and semifluid, somewhat foul-smelling material, containing numbers of large clots. The omentum was rolled and lightly applied transversely to the anterior abdominal wall, thus confining most of the material to the left upper quadrant. The intestinal loops were cyanotic, and the serosal surfaces of the diaphragm, liver and mesenterics were deeply hyperemic and in part covered with a thin, dirty brown exudate.

When the mass of material was removed, there was seen, projecting from the left border of the stomach, a thin-walled sac, which after fixation measured 4 by 3 by 3 cm. The upper border of the diverticulum was 1 cm below the esophageal-cardiac junction, and the diverticulum extended for 4 cm along the



Liver raised to show opened esophagus, stomach and duodenum. A white thread passes through the perforation in the diverticulum and lies in the lumen of the stomach and duodenum, reaching the point of atresia. All the structures proximal to the atresia are dilated.

greater curvature, reaching a point 5 cm above the pyloric sphincter. Along the anterior surface of the diverticulum was an irregular rent, of which the ante-mortem dimensions could not be determined because of its extreme friability. After fixation (and with some postmortem tearing) the aperture was 2.5 cm in the greatest diameter. Grossly no muscle could be recognized in the thin membrane forming the wall of the diverticulum.

The lower portion of the esophagus was dilated. After fixation its diameter at the widest point, 15 cm above the cardia, was 2.5 cm. The stomach was moderately distended, the pyloric sphincter relaxed and wide. The first portion of the duodenum was markedly distended. In the fixed state its greatest circumference was 4.5 cm. Two and five-tenths centimeters below the pyloric sphincter

the duodenal lumen became suddenly narrowed, and at the level of the ampulla of Vater it was completely occluded. The common bile duct was slightly distended, measuring after fixation 2.5 mm in diameter. A probe readily passed from above to the point of atresia in the duodenal wall but not into the duodenal lumen of either the upper or the lower segment. However, bile was present below the point of atresia, while a chemical test failed to reveal any in the contents above. The duodenum below the point of atresia was of normal diameter. The remainder of the small intestine was slightly narrow and contained light green semisolid material. The large intestine was also of rather narrow diameter and contained semisoft material stained light brown and light green. The intestinal tract showed no other abnormalities.

The bile ducts, except as described, and the gallbladder were normal. The liver appeared normal, without evidence of bile stasis. Multiple cross sections of the pancreas revealed the pancreatic duct not distended but its wall stained a deep green. The gland appeared otherwise normal.

*Microscopic Examination*—Sections were taken through the stomach to include both normal stomach and a portion of the diverticulum. The muscular layer ended abruptly, and in the region of the diverticulum there was present only mucosa, submucosa and serosa. The serosa was covered with an exudate composed of red blood cells, fibrin, necrotic leukocytes and bacteria. The underlying submucosa was edematous and congested but showed no inflammatory exudate. In one section the free end of the muscle had pulled away from the submucosa to form a small protuberance beneath the serosa. It had carried with it part of the submucosa, and in this region there was some hemorrhage. In the same section the mucosa in the region of the diverticulum appeared stretched, and further evidence of this was the thinning of the muscularis mucosae as the edge of the section was approached.

The region of the atresia was studied in serial sections. Longitudinal section showed that the atresia was due to a membrane made up of double layers of normal wall, lying back to back, with some aberrant pancreatic tissue occurring at the base and extending for a short distance between the layers of the membrane. The pancreatic duct and the common bile duct could be traced through this area into the lower segment.

The liver showed no evidence of bile stasis.

Several pancreatic ducts contained brown pigment and bacteria. No inflammatory reaction was present. The ducts were not distended. There was no change in the acinar or islet tissue.

#### COMMENT

The problem arises whether there is any necessary and causal relation between the congenital obstruction of the duodenum and the diverticulum of the stomach. As Martin<sup>1</sup> pointed out in his paper, the most common site for a gastric diverticulum is near the cardiac opening, and it is in this region that the musculature of the stomach is most poorly developed. In the present case the diverticulum is along the greater curvature, somewhat below the cardia. It is most probable that this diverticulum is congenital and that it is independent of the atresia of the duodenum. It is difficult to believe that the obstruction within the duodenum served to raise the intragastric pressure during antenatal life sufficiently to have produced thinning of the stomach wall and to have done so not at a natural point of weakness. Busch,<sup>5</sup> in a study

5 Busch, M. Frankfurt Ztschr f Path 30 30, 1924

of rupture of the stomach, pointed out that the usual site of rupture is along the lesser curvature, close to the cardia. Further, such a diverticulum has never been reported in association with congenital obstruction of the intestine.

However, the dilatation of the duodenum proximal to the point of atresia and the dilatation of the stomach and of the esophagus are evidence of an increase in pressure in this portion of the gastrointestinal tract. It seems reasonable to assume that the increase started with birth and the taking of food. How much of a role antenatal swallowing of amniotic fluid and antenatal secretion of digestive juices may have played is impossible to evaluate. It seems probable that the increase in pressure was the immediate cause of the rupture of the congenital diverticulum and that this was signaled clinically by the appearance of blood-streaked vomitus.

The only mention in the literature of a case in which congenital stenosis of the duodenum caused rupture proximally is that of Cannon and Halpert<sup>6</sup>. They described, in an 8½ year old girl, partial duodenal stenosis caused by an incomplete membrane at the level of the entrance of the common bile duct, with hypertrophy of the wall of the stomach secondary to the stenosis. In the membrane was a slitlike orifice. Acute obstruction of the lumen was produced by ingested vegetable leaves. Rupture of the stomach followed in several hours. The rupture occurred 1 to 2 cm. from the cardia and was brought about by unusually vigorous treatment with irritant enemas of excessive amount.

#### SUMMARY

A congenital diverticulum of the stomach associated with atresia of the midportion of the duodenum is reported. Rupture of the diverticulum occurred on the sixth day of life. No previous case of a ruptured gastric diverticulum has been found recorded in the literature.

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6 Cannon, P. R., and Halpert, B. Arch. Path. 8: 611, 1929.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—In the school of medicine of the University of Colorado, W C Black has been promoted to professor and K. T. Neuberger to assistant professor of pathology, R M Mulligan has been appointed instructor in pathology and A S Lazarus instructor in bacteriology

C F Graham has been appointed associate professor in the department of bacteriology and pathology in Albany Medical College

Edgar H Norris, professor of pathology in the college of medicine of Wayne University, Detroit, has been elected dean of the college

As provided by recent legislation, Perry J Melnick has been appointed pathologist to the cancer diagnosis division of the department of public health of the State of Illinois

C C Bass, dean and professor of experimental medicine in the school of medicine of Tulane University, New Orleans, has retired, having reached the age limit

**Awards**—The Eli Lilly Award of a medal and \$1,000 was presented to John G Kidd, of the Rockefeller Institute for Medical Research, by the Society of American Bacteriologists at its last annual meeting This award is given for outstanding work in bacteriology or immunology by a person under 31 years of age

At the meeting of the American Society of Tropical Medicine on Nov 21, 1939, the Walter Reed medal was awarded to William B Castle, professor of medicine, Harvard Medical School, Boston, for work on sprue and related anemias

**Society News**—The annual meeting of the Society of American Bacteriologists was held in New Haven, Conn, Dec 28-30, 1938 This was the fortieth anniversary of the society, which was founded in New Haven Charles Thom, in charge of soil microbiology in the United States Department of Agriculture, was elected president

**International Salmonella Center**—This center, established at the State Serum Institute in Copenhagen, supplies Salmonella centers elsewhere with the cultures and serums necessary for diagnosis Thirty-seven such centers have been established These centers will study doubtful cultures of Salmonella without any charges The centers in this country are Beth Israel Hospital (Dr F Schiff), New York, New York State Department of Health (Dr A B Wadsworth), Albany, N Y, University of Kentucky (Dr A R Edwards, department of animal pathology), Lexington, Ky

**Microfilm Sets of Periodicals**—The Committee on Scientific Aids to Learning (President Conant of Harvard, chairman) has made a grant to cover the cost of making a microfilm master negative, on the most expensive film, of sets of volumes of scientific and learned journals This permits the nonprofit Biblionfilm Service to supply microfilm copies at the sole positive copy cost, namely, 1 cent per page for odd volumes and ½ cent per page for any properly copyable ten or more consecutive volumes The number of pages will be estimated on request to American Documentation Institute, care of offices of Science Service, 2101 Constitution Avenue, Washington, D C

**Legislation for Blood Grouping Tests**—Laws providing for blood grouping tests in cases of disputed parentage are now in force in New York, Wisconsin, Ohio and New Jersey It has been recommended that laws of this kind should include provision for the payment of experts for their services in such cases by the jurisdiction concerned, because in so many instances the men involved are unable to pay any fees

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

THE EARLIEST KNOWN REFERENCE TO THE HEART AND CIRCULATION W W HAMBURGER, *Am Heart J* **17** 259, 1939

This is a review of "the earliest known reference to the heart and circulation" as recorded in the Edwin Smith Surgical Papyrus, of about 3,000 B C The paper is of interest because it illustrates "how very distant and far reaching are the roots of our knowledge in the biological sciences"

EFFECTS OF DIPHTHERIA TOXIN ON THE HEART R W BOYLE, C H McDONALD and A F DeGROAT, *Am Heart J* **18** 201, 1939

The hearts of 7 dogs which had been given varying doses of diphtheria toxin were studied with respect to electrocardiographic behavior, distribution of glycogen and pathologic changes The electrocardiographic changes noted consisted of various degrees of block and abnormalities of the T wave Parenchymatous degeneration of a mild degree was invariably present The most characteristic change was the presence of hyaline streaks scattered through the muscle fibers Glycogen—taking the heart as a whole—showed a 10 per cent rise in the hearts of dogs given a slowly lethal injection of diphtheria toxin A rise was noted in the two atria and the two ventricles, and no change in the septum A quickly lethal dose failed to produce any effects on cardiac glycogen In view of the increases in cardiac glycogen which occur during starvation and of the self-imposed starvation (anorexia) always present in severe diphtheritic toxemia, it is suggested that the moderate rise encountered in such cases is the result of starvation rather than of any specific effect of the diphtheria toxin on cardiac glycogen

FROM AUTHORS' SUMMARY

MINERAL APPETITE OF PARATHYROIDECTOMIZED RATS C P RICHTER and J F ECKERT, *Am J M Sc* **198** 9, 1939

Parathyroidectomized rats showed a markedly increased appetite for solutions of calcium salts (lactate, acetate, gluconate and nitrate) and an aversion toward a solution of disodium phosphate (dibasic sodium phosphate) Parathyroidectomized rats also showed an increased appetite for solutions of strontium and magnesium salts These results agree with present knowledge concerning the disturbance of calcium and phosphorus metabolism following parathyroidectomy The decreased mortality and the alleviation of symptoms of deficiency in parathyroidectomized rats given access to solutions of calcium salts add further evidence that rats have an ability to make selections conducive to their well-being

FROM AUTHORS' SUMMARY

EFFECT OF DIET ON NEPHRITIS IN RATS J E SMADEL and L E FARR, *Am J Path* **15** 199, 1939

In rats of the so-called Whelan strain the chronic nephritis which follows the administration of antikidney serum can be markedly influenced by isocaloric diets containing different proportions of protein and carbohydrate

FROM AUTHORS' CONCLUSIONS

EXPERIMENTAL PRODUCTION OF NEUTROPENIA WITH AMINOPYRINE E M BUTT,  
A H HOFFMAN and S N SOLL, Arch Int Med **64** 26, 1939

It has been shown in these experiments on dogs that aminopyrine has a toxic effect on the bone marrow when administered orally in large doses. The end result is one of severe aplasia of the marrow rather than of hyperplasia with "maturation arrest." Neutropenia was noted in 1 of the 2 dogs having markedly aplastic bone marrow. A summary of the literature on the experimental production of malignant neutropenia is presented.

FROM AUTHORS' SUMMARY

CAUSES OF THE CESSATION OF GROWTH OF FIBROBLASTS IN EMBRYO JUICE L E  
BAKER, J Exper Med **69** 625, 1939

Experiments designed to ascertain the reason for the cessation of growth of cardiac fibroblasts when they are cultivated in a plasma coagulum with embryo juice as nutrient fluid have shown that it is due, first, to the gradual removal of serum from the coagulum and, second, to an insufficiency of embryo juice. In a medium containing embryo extract at 66 per cent concentration and serum at 8 per cent concentration growth continued until the entire coagulum in a 35 cm flask was covered with tissue. The serum is needed to furnish additional nutriment and also to prevent digestion of the coagulum.

FROM AUTHOR'S SUMMARY

ENHANCING EFFECT OF AZOPROTEINS ON LESIONS A CLAUDE, J Exper Med  
**69** 641, 1939

It is known that solutions of azoproteins, like testicular extracts, possess the property of causing particles to spread through the dermis. The present work shows that azoproteins exhibit, like testicular extracts, the power to increase the size of the lesions caused by virus in the skin of rabbits and the size of tumors in chickens. The results indicate that the extent of the lesion is roughly proportional to the spreading power of the solution. This suggests that the spread of the infective material over a larger area of skin is directly responsible for the enhancing effect. The production of extensive lesions by means of spreading agents may have a practical value when large amounts of working material are needed.

FROM AUTHOR'S SUMMARY

EXPERIMENTAL STUDY OF ATROPHY OF TISSUES AND ORGANS G CAVALLI, Ann  
d'anat path **13** 691, 1936

In three groups of rabbits (1) the renal artery, (2) the renal vein and (3) both the artery and the vein were ligated on one side, and the subsequent changes were studied histologically at weekly intervals. At first there was marked swelling of the kidney, especially when the renal vein alone was tied, due to edema and interstitial hemorrhage. Shrinkage then began, and the kidney became progressively smaller and firmer. At the second week marked degeneration of the tubular epithelium was seen, followed by resorption of necrotic material and organization of the kidney by appearance of granulation tissue and proliferation of connective tissue. Many small newly formed blood vessels were seen, apparently derived from collateral sources, i. e., capsular, adrenal, ureteral and lumbar branches. There were no regenerative processes. In the third week fibrosis was advanced, and metaplastic bone formation began. Possible reasons for the occurrence of metaplastic ossification are advanced. The article also contains a historical survey of the subject of ligation of renal vessels.

PERRY J MELNICK

COMPARATIVE STUDY OF PULMONARY LESIONS OF NERVOUS ORIGIN J TINEL,  
G UNGAR and J BRINCOURT, Ann d'anat path **13** 898, 1936

The lungs of dogs and rabbits were examined after section and after section plus electrical stimulation of the vagus nerves, phrenic nerves and carotid sinus.

Section of the vagus nerves even without electrical stimulation, resulted in a picture resembling bilateral bronchopneumonia, with hepatization and with the presence of an abundant cellular exudate in the alveoli. Electrical stimulation of the phrenic nerves or of the posterior cervical nerve roots or of the carotid sinus resulted in marked edema and hyperemia of the alveolar walls and extravasation of red cells, a picture resembling hemorrhagic infarcts. It is suggested that the nervous system may play a role in the production of pulmonary edema and congestion, pneumonia and pulmonary infarcts.

PERRY J MELNICK

NEPHROGENIC OSTIODYSTROPHY E RUTISHAUSER, *Ann d'anat path* **13** 999, 1936

By inserting many glass needles or copper wires into the kidneys of rabbits Rutishauser was able to produce chronic nephritis of a type which causes a disturbance of calcium and phosphorus metabolism and results in osteodystrophy resembling von Recklinghausen's osteitis fibrosa. Ten rabbits were used. One of these is described in detail as an example. Before operation the blood calcium was 11.3 and the blood phosphorus 6.4 mg per hundred cubic centimeters. Ninety days later the figures were 16.7 and 4.5, and one hundred and thirty-eight days later, 16.9 and 6.2, respectively. One hundred and forty-nine days after operation the rabbit died in a state of emaciation. At autopsy there was found chronic cicatrizing nephritis about the glass needles. Calcium deposits (calcium "metastases") were found in the renal tubules, stomach and lungs (i.e., in areas where there was local alkalosis consequent to the excretion of acid substances). The bones were found partially decalcified, and the microscopic picture was that of osteitis fibrosa. The parathyroid glands were somewhat hypertrophied.

PERRY J MELNICK

ORIGIN OF OBLITERATING ARTERITIS AND ITS PRODUCTION FOLLOWING REPEATED GRAFTING OF ADRENAL GLANDS R LERICHL and F FROELICH, *Ann d'anat path* **13** 1039, 1936

Adrenal glands were repeatedly grafted into 5 rabbits, from 6 to 40 grafts were performed over periods varying from twenty-seven days to six months and seven days. In all of these animals changes were found in the arteries on microscopic examination. These changes consisted chiefly of marked thickening and hypertrophy of the intima and media, leading to stenosis and sometimes to complete obliteration of the lumen. The findings in each of the 5 animals are described in detail. The authors conclude that the adrenal gland exerts an influence in the production of obliterating arteritis.

PERRY J MELNICK

## Pathologic Anatomy

CEREBRAL VASCULAR DISEASE ACCOMPANYING SICKLE CELL ANEMIA W H BRIDGERS, *Am J Path* **15** 353, 1939

A study of 2 patients with sickle cell anemia (the first was an adult and the second a child) has shown that the disorder may first become manifest through the appearance of signs and symptoms indicative of cerebral vascular disease. The clinical features in such patients lead to the diagnosis of either cerebral vascular thrombosis or intracranial hemorrhages. The pathologic changes seen in the first of the patients establish the fact that in sickle cell anemia the large subarachnoid cerebral arteries may undergo gradual obliteration with final complete closure through the operation of a process identical with that which results in occlusion of the splenic arteries. This process is one of endarterial intimal proliferation and not of thrombosis. In the second patient autopsy showed that another vascular process, quite different from endarterial intimal proliferation, also occurs in sickle cell anemia. This process develops in connection with the

small intracerebral vessels and may result in multiple focal necroses and hemorrhages in the brain, in contrast with the large infarcts that characterize the proliferative obstructive process in the larger arteries. The nature of this second process is not clear.

FROM AUTHOR'S SUMMARY

**PATHOLOGIC CHANGES FOLLOWING THERAPEUTIC HYPERTHERMIA** L. LICHTENSTEIN, *Am J Path* **15** 363, 1939

A description is given of the changes observed at autopsy in a case of uncontrollable hyperpyrexia (109 F) ensuing on hyperthermia treatment for arthritis of the finger joints. The hyperpyrexia (which developed in the course of the third of a series of treatments) was associated with coma and respiratory failure, and the patient died about twenty-five hours after the fever was initiated. In this case the significant pathologic changes were (1) multiple punctate hemorrhages and necrobiosis in the gray matter of the cerebral cortex, (2) hemorrhage in the left internal capsule, (3) thrombosis of venules and capillaries in the cerebral cortex and internal capsule, (4) cerebral congestion and edema, (5) infarction of the kidneys and spleen, (6) marked hepatic degeneration and edema, and (7) pulmonary congestion, hemorrhage and edema. The changes found in this case have been correlated with, and discussed in relation to, those in 9 cases previously recorded in the literature. Certain of the changes seen in the case reported here, notably the vascular lesions, have not hitherto been described in connection with fatalities following fever therapy. Specifically there seems to be no previous description of the thrombosis of venules and capillaries in affected portions of the brain and of the infarcts in the kidneys and spleen apparently due to focal necroses of small arterial branches in these organs. The principal complications and sequelae of hyperthermia—especially its effects on the brain, blood vessels and liver—are indicated. Attention is also drawn to the fact that the reactions to therapeutic hyperthermia are sometimes serious even when they are not fatal.

FROM AUTHOR'S SUMMARY

**MYOCARDIAL INFARCTION WITHOUT SIGNIFICANT LESIONS OF THE CORONARY ARTERIES** H. GROSS and W. H. STERNBERG, *Arch Int Med* **64** 249, 1939

Fifteen cases of myocardial infarction without demonstrable occlusion of the coronary arteries were studied. Arteriosclerosis of the coronary arteries was minimal. In 1 case all the major vessels were sectioned serially. A variety of physiologic mechanisms which might account for myocardial damage in the absence of vascular occlusion is outlined. These may be (1) mechanical, (2) reflex or (3) humoral. Mechanical factors include temporary falls in intraaortic blood pressure, tachycardia, phasic variations of the coronary flow and cardiac hypertrophy. Changes either in the number of formed elements or in the viscosity of the blood, such as are seen in anemia and polycythemia, are contributory factors. Reflex factors may produce anoxia of the myocardium from failure of adequate compensatory dilatation of the coronary arteries or from coronary constriction. The stimuli for these reflexes may arise either in the heart and its associated structures or in other parts of the body. Humoral factors and possibly other agents, including acetylcholine, epinephrine and pitressin, also alter the caliber of the coronary arteries. The presence of hypertension in 13 of the 15 cases is of great interest. Vasoconstrictor phenomena are common in hypertension, and it is conceivable that extreme coronary vasoconstriction may have played a role. The underlying cause of the anginal seizure, whether transitory or associated with myocardial infarction, is ischemia of the myocardium. Similarly, the electrocardiographic changes in both instances are also the result of myocardial ischemia. The duration of ischemia will determine whether the cardiographic and myocardial changes are reversible or not. Though the type of case reported is infrequent, it is nevertheless of importance, since similar physiologic factors must also play a role in many cases of ordinary coronary disease and myocardial damage.

FROM AUTHORS' SUMMARY



# HISTOLOGIC CHANGES IN THE NERVOUS SYSTEM IN CASES OF PEPTIC ULCER A R VONDERAHE, *Aich Neurol & Psychiat* **41** 872, 1939

The effect of peptic ulcer (duodenal, gastric or both) on the brain or vice versa was investigated by Vonderahe on extensive clinicopathologic material. In the 14 cases of peptic ulcer studied, hemorrhages, frequently punctate, were found in certain areas of the diencephalon (thalamus, hypothalamus) and in the dorsal motor nucleus of the vagus nerve, usually on one side. Control studies of the diencephalon (in cases of malignant hypertension, puerperal septicemia, paresis, carcinoma of the lungs and diabetes mellitus) disclosed no evidence of hemorrhages into the aforementioned regions, though such hemorrhages were found in association with other conditions, especially irritative disease of the abdominal viscera and peritoneum and, in some cases, diabetes mellitus. On the whole, hemorrhages in specific areas of the thalamus and hypothalamus are, according to Vonderahe, a consistent observation in cases of peptic ulcer. The medulla was investigated in a variety of pathologic conditions (disease of the heart, kidneys, lungs and other organs), and neither hemorrhages nor diapedesis was found to occur in the motor nucleus of the vagus nerve. Only in the presence of peptic ulcer were punctate hemorrhages disclosed with regularity in this nucleus. In 20 per cent of cases of tumor of the brain there was evidence of incipient or well advanced gastrointestinal ulceration, and in 11 of 56 cases of gastrointestinal ulceration gross non-neoplastic lesions of the brain and the meninges were present. The hemorrhages are supposed to be due to vasomotor alterations in the cerebral blood vessels as the result of implication and excessive stimulation of the visceral afferent components of the vagus and sympathetic nerves.

GEORGE B HASSIN

# HISTOLOGIC STUDY OF OSLER'S NODES L CORNIL, M MOSINGER and A X JOUVE, *Ann d'anat path* **13** 675, 1936

The authors made a histologic study of a subcutaneous rheumatic node removed twenty-four hours after its appearance. The essential finding was an injury of the endothelium of the smaller blood vessels. Swelling, vacuolation and sometimes necrosis of the endothelium were seen, with occasional secondary thrombosis. Usually, however, proliferation of the endothelial cells occurred, sometimes sufficient to occlude the lumen. Necrosis about the blood vessels and marked cellular infiltration of the adventitia were also seen. Bacteria were not found. The authors do not believe that these lesions are embolic but consider them as of toxic or hyperergic origin, and they compare them to Fraenkel's nodules in typhus fever and to the rose spots of typhoid. (The descriptions and photomicrographs give the impression, in the light of recent concepts regarding the nature of rheumatism and in view of the work of Rossle, Klinge and many others, that these lesions should probably be considered as allergic granulomas.)

PERRY J MELNICK

# CYTOLOGY OF THE VASCULAR AND MESENCHYMAL LESIONS IN TYPHUS FEVER AND THEIR SIGNIFICANCE E CRACIUN, *Ann d'anat path* **13** 817, 1936

In typhus fever the cutaneous nodules, which occur about the arterioles in the reticular layer of the dermis, first described by Fraenkel, have a specific histologic development. This consists in an endothelial injury (sometimes necrosis and sometimes proliferation and desquamation into the lumen), followed by a histiocytic infiltration of the wall of the blood vessel and of its adventitia. The specific character of Fraenkel's nodules has been confirmed by many. In this article Craciun, on the basis of a study of the observations made at 22 autopsies, concludes that this same specific character is found in the visceral lesions of typhus fever also. He gives a detailed description and discussion of the visceral lesions.

PERRY J MELNICK

ANATOMIC STUDY OF INTERSTITIAL ANEURYSMS OF THE LUNG M DELHOMME  
and M IGLESIAS Y BETANCOURT, *Ann d'anat path* **13** 961, 1936

Of 4 cases of pulmonary tuberculosis in which death followed hemoptysis and in which an autopsy was made, 3 were studied in detail. The lungs were found to contain large hematomas enclosed in cavities. These cavities, however, had all the characteristics of aneurysms, having lamellar layers of fibrin at the periphery and afferent and efferent vessels. The authors present a theory of formation of these interstitial aneurysms on the basis of caseous involvement of a blood vessel with weakening of part of the wall and formation of an aneurysm.

PERRY J MELNICK

CENTERS OF ORIGIN AND MODES OF DEVELOPMENT OF VENOUS THROMBOSIS OF  
THE LOWER EXTREMITY R NEUMANN, *Virchows Arch f path Anat* **301**  
708, 1938

In 165 consecutive necropsies the venous system was thoroughly examined for thrombosis. Special attention was paid to the veins of the lower extremities, and the observations form the basis of this report. Eighty-four of the subjects were women. Eighty-one were men. The youngest was 17 years old, the oldest 88. Thrombosis was detected in 100 of the 165 subjects. The localization was as follows: plantar region, 71 per cent, internal malleolar region, 17 per cent, leg, 87 per cent, thigh, 22 per cent. Thrombosis limited to the veins of the thigh alone was not observed. Great importance is attached to the fact that the malleolar vessels, which connect the veins of the foot and the leg, were so infrequently involved even when both the plantar and the leg regions were seats of thrombosis. This leads to the conclusion that there are two independent centers of origin of thrombosis in the veins of the lower extremity: one in the plantar region, the other in the leg region. Two clinical types are recognized, depending on the center of origin: (1) a benign type, which has its center of origin in the veins of the leg and which is characterized by slow progression, an incidence that increases progressively with age and a tendency toward the occurrence of multiple nonfatal emboli in the lung, (2) a malignant type, which has its center of origin in the plantar region and is characterized by rapid progression of the thrombosis, occurrence at earlier ages, with no increase with age, and a tendency toward fulminating fatal embolism of the lung. In both types the source of emboli is a thrombus that has progressed to the veins of the thigh. In progressive ascending thrombosis segmentation results where ligaments, tendons and the like cross over veins. In thrombosis three factors are recognized: (1) a preparatory factor, usually endovascular, (2) a precipitating factor, which may be vascular, endovascular or perivascular, and (3) a localization factor, which is usually perivascular.

O T SCHULTZ

ADAPTIVE CHANGES IN THE CRANIUM IN RELATION TO BRAIN GROWTH J  
ERDHEIM, *Virchows Arch f path Anat* **301** 763, 1938

This article, written in Erdheim's lucid style, had evidently been completed before his death. He describes in detail the changes that occur in the skull in its growth and in its adaptation to the brain from fetal life to old age. It is constantly in a state of flux, which makes of the cranium a plastic structure in spite of its osseous nature. Three processes are interrelated and are in constant interplay with each other. These Erdheim terms *Anbau*, *Abbau* and *Umbau*, words that do not lend themselves to translation into single terms. They might be translated as "construction, or building," "destruction, or absorption," and "reconstruction, or rebuilding," although these words do not quite express the shades of meaning of the original. In these processes the dura, the epicranial periosteum and the endosteum of the diploe play a part. As the internal surface of the bone is resorbed by the dura, new bone is laid down by the diploe and epicranium. Increase in the size of the cranial cavity is not a simple matter of

expansion and growth of bone at the suture lines but a progressive rebuilding, so that the inner and outer tables and the diploe occupy a position relatively distant from that which they had in earlier life. Increase in the area of the flat bones occurs by a similar process of resorption and new formation of bone at the suture margins. Resorptive and reconstructive changes induced by the dura lead to modeling of the internal surface of the cranium. The rate at which the various processes proceed varies, and at times growth ceases, resulting in the formation of lines of cessation of growth, to which Erdheim had attached importance in previous communications. That the inner surface of the cranium is not modeled or molded to correspond to the sulci and convolutions of the surface of the brain is due to the fact that the pia-arachnoid with its subjacent fluid forms a smooth envelop which protects the dura against the apparent inequalities of the cerebral surface. Having established what he considers the normal course of events, Erdheim proceeds to discuss in three final sections three pathologic states in the light of his concept. In senile hyperostosis the volume of the cranial cavity must decrease to accommodate itself to the shrinking senile brain. An increase in the quantity of subdural liquid plays a part, but appositional bone formation by the dura is more important. Next the changes in the skull associated with increased intracranial pressure are described, especial attention being paid to the reconstructive alterations that occur when the pressure is permanently restored to normal by removal of the cause of the increase in pressure. The final section is devoted to *Luckenschadel*, in which the cranial juga are greatly increased and thickened and the digitate impressions are correspondingly widened and the bone over them thinned, to such a degree that perforation of the skull may occur. It appears that Erdheim had contemplated a study along similar lines of other pathologic changes in the cranial bones.

O T SCHULTZ

### Microbiology and Parasitology

VIRUS PNEUMONIA OF INFANTS SECONDARY TO EPIDEMIC INFECTIONS E W GOODPASTURE and others, *Am J Dis Child* **57** 997, 1939

Five cases of a hitherto undescribed virus infection of the lungs of infants, which develops especially following measles and whooping cough, are recorded. The virus invasion usually appeared to be secondary and tended to pave the way for bacterial infection of the lungs. The presence of the virus was indicated by the occurrence of nuclear inclusions in epithelial cells of the trachea and bronchi and their mucous glands and in the alveolar epithelium. The affected cells rapidly became necrotic, with resultant ulceration of surfaces. The virus appeared to be different from that of herpes simplex and from the agent of the so-called inclusion disease of infants. Experimental inoculation of the infected lung tissue into rabbits, mice, opossums, chicken embryos and a *Macacus rhesus* monkey failed to establish the infection.

FROM AUTHORS' SUMMARY

REACTION OF KILLED TUBERCLE BACILLI C T OLCOTT, *Am J Path* **15** 287, 1939

When heat-killed tubercle bacilli are injected into the peritoneal cavities of rabbits, tubercles and tuberculous tissue form more quickly and become more advanced in animals that have received preliminary intracutaneous or subcutaneous injections of killed tubercle bacilli. The difference is greatest three or four weeks after the intraperitoneal injection of the bacilli, when the lesions in prepared and in control animals are at their height. After five weeks no difference in the two groups is evident, and later the lesions in both regress. The extent of tuberculous lesions is greater in the prepared animals, which are both immunized and sensitized, than in their controls, but there is no close correlation between sensitization as measured by the tuberculin reaction and the extent of the tuberculous lesions. Heat-killed tubercle bacilli cause necrosis with the characteristics of caseation.

both in control (unprepared) animals and in those that have received preparatory injections, but this type of lesion is more frequently found in the latter. Caseation is in general more advanced in sensitized animals, but there is no exact correlation between sensitization and caseation. When killed tubercle bacilli have been injected into the peritoneal cavity, tubercle-like nodules composed of epithelioid cells are often found in the retrosternal lymph nodes, spleen, liver and lungs of both immunized and control animals.

FROM AUTHOR'S SUMMARY

SUBAXILLARY GLAND VIRUS OF GUINEA PIG C. T. ROSENBUSCH and A. M. LUCAS, *Am J Path* **15** 341, 1939

There is some evidence that young guinea pigs are more resistant to the action of the subaxillary gland virus than full-grown animals of the same stock.

FROM AUTHORS' SUMMARY

INDUSTRIAL DUSTS AND THE MORTALITY FROM PULMONARY DISEASE A. J. LANZA and R. J. VANE, *Am Rev Tuberc* **39** 419, 1939

Early studies of occupational mortality focused attention on dust as a cause of respiratory diseases. Virtually all kinds of dusts were implicated. Present day studies point to the serious damage of lung tissue caused by a few dusts, notably silica and asbestos, and to the relatively little damage caused by many other dusts. Yet only qualified general conclusions are permitted except for silica. The death rate from tuberculosis among occupational groups who are freely exposed to silica so far exceeds those found for other groups as to leave little room for doubt that silica is implicated. With regard to American mortality from silicate and other inorganic dusts not containing free silica, data are meager. The effects of aluminum oxide, silicon carbide and other substances used in manufactured wheels are slight. British data show lower than average mortality from tuberculosis up to the age of 35, but substantially higher mortality from this disease after the age of 45, for a group of men exposed to inorganic dusts other than silica dust. The relationship between the inhalation of dust and acute pulmonary disease remains a field for further investigation.

H. J. CORPER

BLASTOMYCOSIS D. B. MARTIN and D. T. SMITH, *Am Rev Tuberc* **39** 488, 1939

American blastomycosis is a distinct clinical entity, caused specifically by *Blastomyces dermatitidis*. Two types of infection caused by this fungus are recognized clinically: (a) cutaneous blastomycosis, a chronic or subacute ulcerating process, usually responding to treatment with iodides or radiation, and (b) systemic blastomycosis, a highly fatal disease, characterized by pulmonary infection and widespread distribution of lesions. The disease is more common in males. The serum of heavily infected patients discloses the presence of antibodies. In some patients a condition of hypersensitiveness to the fungus develops, which diminishes in the terminal stages of the disease. The degree of hypersensitiveness can be estimated by cutaneous tests and is materially reduced by repeated injections of minute doses of heat-killed vaccine. In some cases potassium iodide is curative, but it is a dangerous drug to administer to patients allergic to the fungus. In systemic blastomycosis iodide therapy should be started only after the state of hypersensitiveness has been excluded by cutaneous tests or artificially reduced by therapeutic injections of vaccine.

H. J. CORPER

EXPERIMENTAL AND CLINICAL GRANULOMA INGUINALE R. B. GREENBLATT, R. B. DIENST, E. R. PUND and R. TORPIN, *J A M A* **113** 1109, 1939

Granuloma inguinale was experimentally reproduced in 3 human beings but failed to develop in 1. It failed to develop in laboratory animals in spite of repeated attempts to reproduce it. When the disease was reproduced, the course

was comparable in every way to that of the spontaneous type. Donovan bodies were recovered to the exclusion of other organisms from the pseudobuboes that developed in the 3 patients. The incubation period could not be determined, however, the classic picture of the disease was complete in about fifty days. This is the first instance in which granuloma inguinale was experimentally produced in a human being by the use of an exudate which contained only the Donovan bodies and no other demonstrable organisms.

The pseudobubo that so frequently follows a primary focus on the external genitalia is not adenitis per se but a subcutaneous granuloma. Histologic study of regional and underlying lymph nodes revealed only moderate endothelial hyperplasia. However, of 2 patients Donovan bodies were demonstrated in the underlying cervical and inguinal lymph nodes in one, who also had extragenital involvement, and in one regional inguinal node in the other. Such observations prove that the Donovan body can and does travel by way of the lymphatics. The hypothesis is presented that Donovan bodies may reach the lymph nodes, where temporary though mild focal reactions with perilymphadenitis occur. During this process Donovan bodies may reach the papillae and corium of the overlying skin and set up a subcutaneous granuloma. Here the process may be subacute, resulting in a subcutaneous abscess, or may be chronic and a massive granulomatous tissue causes the overlying epidermis to bulge—hence the pseudobubo, for prior to rupture and the burgeoning of the typical raised granulations it simulates the bubo of the other venereal diseases.

The nature of the Donovan body remains an enigma to most students of the subject. Contrary to the many reports on the isolation and culture of an organism comparable to the Donovan body, it is doubtful whether the causal agent of granuloma inguinale has ever been cultivated. Such cultivated organisms on inoculation into human beings have failed in every instance to reproduce the disease. The method of reproduction in mononuclear endothelial cells and the growth requirements of the organism as well as the clinical behavior of the disease lead the authors to assume that the Donovan body is a sporezoan.

#### FROM AUTHORS' SUMMARY

#### MODE OF ACTION OF SULFANILAMIDE ON STREPTOCOCCUS F. P. GAY and others, J. Exper. Med. 69: 607, 1939

The precise mode of the therapeutic action of sulfanilamide on the streptococcus can be arrived at only by considering the sum total of factors that inhibit or favor the natural growth of the micro-organism under the experimental conditions that obtain, whether in vivo or in vitro. Conclusions too sweeping have been drawn from the study of a single variable factor, such as an unfavorable temperature or the absence or the presence of peptone. Gay and his co-workers have attempted here to analyze the factors that have hitherto been recognized and some new ones, but particularly the relationship of these factors to one another. The result obtained on adding sulfanilamide to a culture of the streptococcus in a test tube is usually bacteriostasis and not complete destruction of even small numbers of the bacteria. This is on the condition that the suspending medium is one that is favorable to the growth of the micro-organism, the more growth-promoting the medium is the less the bacteriostasis. If, on the other hand, the medium is too poor or is one that in itself inhibits growth, the addition of sulfanilamide may lead to sterilization of the culture. The conditions for growth of the streptococcus in the body of the rabbit or the mouse depend on the strain of bacteria used, but are on the whole favorable. Defense, however, in the form of phagocytosis by both polymorphonuclear and mononuclear cells is attempted even in the susceptible animal. When sulfanilamide is used to treat such an animal, or when sulfanilamide-grown (inhibited) streptococci are employed, phagocytosis is pronounced, whether studied in the test tube or in the animal body. In the rabbit the delay in streptococcal activity in the presence of sulfanilamide and the resultant increase in phago-

cytosis by polymorphonuclears allow mononuclear cells to accumulate, and recovery may result. Sulfanilamide not only does not completely destroy the streptococcus but does not even impair its innate virulence. It acts on the streptococcus not only by inhibiting growth but by temporarily inhibiting the formation of hemotoxin, but only under certain conditions. The drug does not neutralize hemotoxin already formed. No significant effect of sulfanilamide on the formation of leukocidin or fibrinolysin by the streptococcus has been evident in these experiments. Sulfanilamide differs in one important respect from other drugs that are destructive of protozoa and bacteria either in the test tube or in the body. Protozoa fix or adsorb arsenicals and acriflavine, which kill them variably in vitro and in vivo. Streptococci fix both gentian violet and acriflavine, which have marked destructive action in the test tube but are less effective in vivo. Sulfanilamide is not diminished at all by contact in vitro with large masses of streptococci, nor does its action render these streptococci more capable of absorbing gentian violet or acriflavine than untreated streptococci, to be destroyed by those highly bactericidal substances.

## FROM AUTHORS' CONCLUSIONS

BACTERICIDAL AGENT FROM SOIL BACILLUS R J DUBOS, J Exper Med **70** 1, 11 and 249, 1939

A gram-positive, spore-bearing aerobic bacillus capable of lysing the living cells of many gram-positive microbial species has been isolated from soil. Cultures of this bacillus in peptone mediums release during autolysis a soluble agent which exerts a bactericidal effect on all the gram-positive micro-organisms so far tested and inactivates their dextrose dehydrogenases. It also inhibits the growth of the susceptible species in culture mediums. Several of the gram-positive species when incubated with the bactericidal agent undergo lysis. It appears, however, that the lysis is only a secondary process, due to the autolytic enzymes of the susceptible cells, and that it follows on some other primary injury caused by the active agent. The bactericidal agent is ineffective against all the gram-negative bacilli so far tested.

Dubos and C. Cattaneo show that the same agent protects white mice against infection with large numbers of virulent pneumococci. It also exerts a curative effect when administered to mice several hours after injection of the infective organisms. The minimal effective dose of the bactericidal agent and the degree of protection afforded are approximately the same for all virulent pneumococci, irrespective of type specificity. The bactericidal agent is entirely ineffective against infection with virulent Friedlander bacilli (type B). This agrees with the fact that the agent does not affect gram-negative bacilli in vitro. The protective action exerted by the bactericidal agent against experimental pneumococcal infection depends on the same mechanism which determines its bactericidal effect in vitro.

A cell-free extract of cultures of an unidentified soil bacillus which exerts a bactericidal effect on gram-positive micro-organisms has been described in previous reports, the first active preparations which were obtained were found to contain a protein precipitable at  $pH$  4.5. In the present report it is shown that the bactericidal agent can be obtained in an active form free from protein. The purified preparations retain all the activity of the original material, both in vitro and in vivo.

## FROM AUTHOR'S SUMMARIES

A TYPE OF GROUP A HEMOLYTIC STREPTOCOCCUS WHICH FAILS TO FORM PEROXIDE A T FULLER and W R MAXTED, Brit J Exper Path **20** 177, 1939

Fuller and Maxted note that previous observations on the formation of peroxide by hemolytic streptococci have been without reference to the group or the type of the strain. They show that all known types of hemolytic streptococci of group A form peroxide except type 3. It is remarkable that strains of the latter type, which produce no peroxide in aerobic culture, are inhibited by catalase in the medium.

THE SIZE OF THE VIRUS OF LYMPHOCYTIC CHORIOMENINGITIS AS DETERMINED BY ULTRAFILTRATION AND ULTRACENTRIFUGATION T F M SCOTT and W J ELFORD, Brit J Exper Path **20** 182, 1939

Scott and Elford have sought to determine the particle size of the infective agent of lymphocytic choriomeningitis as it exists in broth extracts of infected mouse brain. The evidence furnished by the two methods applied, namely, ultrafiltration and centrifugation analysis, has indicated the probable diameter of the virus to be 40 to 50 microns.

MILIARY TUBERCULOSIS IN THE PANCREAS OF CHILDREN H W SACHS, Frankfurt Ztschr f Path **51** 63, 1938

Prompted by Ghon, Sachs examined 20 cases of generalized miliary tuberculosis in order to see if there were changes in the pancreas. The histologic examination by serial sections showed that in 70 to 84 per cent of the cases miliary tubercles were present in the pancreas. Most of the tubercles were found in the peripheral portions of the pancreas and in the head of the pancreas.

OTTO SAPHIR

LABORATORY CASES OF WEIL'S DISEASE A WELCKER, Zentralbl f Bakt (Abt 1) **141** 400, 1938

Welcker discusses the problem of laboratory infections by *Leptospira icterohaemorrhagiae*. He summarizes the literature dealing with such accidents and cites numerous reports of cases in which workers have inoculated themselves accidentally with a syringe or have been bitten by rats. He also emphasizes the fact that within recent years these spirochetes have been found in healthy white rats and that several cases of infection from the handling of these animals have been reported. No original data are given inasmuch as the paper is essentially a summary of the problem and is meant to serve as a warning to workers who may run the danger of becoming infected while handling white rats.

PAUL R CANNON

RÔLE OF VITAMIN C IN THE GENESIS OF TUBERCULOSIS IN THE GUINEA PIG K E BIRKHAUG, Acta tuberc Scandinav **13** 45 and 52, 1939

Daily oral administration of crystalline vitamin C significantly inhibits the tuberculin reaction in tuberculous guinea pigs. The degree of inhibition is definitely correlated with the urinary excretion and adrenal content of vitamin C.

Hypervitaminosis C induced by daily oral administration of 10 mg of l-ascorbic acid caused a significant increase in body weight and reduction in the invasive lesions and development of generalized tuberculosis in guinea pigs which had been inoculated subcutaneously with approximately 500 viable bovine tubercle bacilli each and killed sixty-four days after inoculation.

A histologic study revealed less of caseonecrotic lesions, more collagenous tissue within and around the tuberculous centers and less dispersion of tubercle bacilli in animals with hypervitaminosis C than in the controls.

FROM AUTHOR'S SUMMARIES

## Immunology

ANTIGENICITY OF THE VIRUS OF TRACHOMA L A JULIANELLE, Am J Path **15** 279, 1939

Clinical observation reveals little if any immunity to trachoma. It has not been possible to demonstrate increased resistance to experimental trachoma in

monkeys following recovery from the infection. The serum or defibrinated blood of patients with active infections of varying duration exerts no neutralizing or protective effect on the virus of trachoma. The serum of infected or recovered monkeys contains no antibodies demonstrable by the usual methods of protection. The serum of rabbits or susceptible monkeys receiving repeated intravenous injections of active trachomatous tissues contains similarly no antiviral substances. It is concluded that the virus of trachoma is an impotent and ineffectual antigen.

FROM AUTHOR'S SUMMARY

SEROLOGICAL SPECIFICITY OF PEPTIDES. K. LANDSTEINER and J. VAN DER SCHEER, *J. Exper. Med.* **69** 705, 1939.

Experiments are described dealing with immune serums to pentapeptides and peptide amides. Absorption and inhibition tests gave no indication of the presence in the immune serums of special antibodies for portions of a peptide molecule, but the antibodies appeared to be specific for an entire pentapeptide even though the serums contained qualitatively different fractions. Marked disparity was found between the reactions of peptides and corresponding amides, indicating differences between acid and other polar groups in their influence on serologic specificity.

FROM AUTHORS' SUMMARY

SENSITIZATION WITH CHEMICAL COMPOUNDS. K. LANDSTEINER and M. W. CHASE, *J. Exper. Med.* **69** 767-1939.

Experiments are described on the period of latency in sensitization to poison ivy and on the time the agent must remain in contact with the skin. The chief matter of investigation concerned the manner in which the whole skin becomes sensitive following treatment at a particular site, especially whether this sensitization is effected by way of the epidermis. Two methods were used to interrupt the continuity of the skin, one that of cutting through both the skin and the underlying thin muscular layer, the other that of removing a strip of skin so as to spare the cutaneous muscle. These procedures led to different results when an extract of poison ivy was applied to the areas thus isolated. With the first method sensitization was mostly prevented, whereas with the second method generalized hypersensitiveness occurred almost uniformly. An explanation is to be found in the severance of the lymph vessels lying on the surface of the muscular layer, pointing to the necessity of a free passage of lymph. On the other hand, the experiments prove that general sensitization is not dependent on maintaining the integrity of the skin around a treated area. An inhibition of sensitization by incisions extending through the panniculus carnosus was seen to some extent in anaphylactic sensitization with protein antigens, namely, when sufficiently small amounts were employed.

FROM AUTHORS' SUMMARY

SEROLOGICAL STUDIES OF REPTILIA. G. C. BOND and N. P. SHERWOOD, *J. Immunol.* **36** 1, 11, 1939.

Isohemagglutination was not observed, but heterohemagglutination and heterohemolysis occurred between genera and between families of the Serpentes, but they showed no relation to zoologic grouping. Snake red blood cells did not contain agglutinogens comparable to human A and B, but some snake serums contained species-specific, and a few contained group-specific, agglutinins capable of reacting with human red cells. These serums when properly absorbed can be used reliably to determine the blood groups of human red cells. Each of 38 specimens of undiluted fresh snake serum hemolyzed both human and sheep red cells, the titer for the latter being considerably higher. The hemolytic action was found to be due to a normal hemolysin and to an excess of complement, which had



a titer, and which reacted to heat and in storage, similarly to guinea pig complement. Snake complement could be used in bacterial and in syphilitic complement fixation tests. That it was not identical with guinea pig complement was demonstrated by the fact that the latter could not take its place in the hemolysis of sheep red cells by strongly hemolytic snake serums.

I DAVIDSOHN

**NONSPECIFIC "DESENSITIZATION" THROUGH HISTAMINE** L. FARMER, *J. Immunol.* **36** 37, 1939

Young virgin guinea pigs were sensitized with horse serum and after from thirteen to fifteen days were given repeated intra-abdominal injections of histamine in increasing amounts. From twenty-six to thirty-six days after sensitization their uteri were tested in the Schultz-Dale experiment. Much larger amounts of horse serum were needed to bring about anaphylactic contraction (with 78 per cent a concentration of more than 1:250,000) in these uteri than in controls, with which the concentration needed was only 35 per cent. Injections of histamine into 9 guinea pigs caused them to become less sensitive to the same substance. The results suggest that histamine is the substance responsible for the anaphylactic contraction of the smooth muscle and thus they support Dale's hypothesis.

I DAVIDSOHN

**SERUM SICKNESS IN RABBITS** M. S. FLEISHER and L. R. JONES, *J. Immunol.* **36** 511, 1939

Horse immune serum to which proper amounts of sodium hydroxide had been added was heated at an optimum temperature for established periods of time and then neutralized with hydrochloric acid. The product retained the major part of the antibody but failed to produce manifestations of serum disease in rabbits. A definite quantitative interdependence was observed among the three factors: alkali, temperature and time of heating. There is evidence to the effect that concentrated pseudoglobulin can be similarly treated, effecting destruction of the factor causing serum sickness without excessively injuring the antibody. However, the toxic manifestations that were observed in some rabbits prohibit at present the application of this method of eliminating the factor causing serum sickness preliminary to the use of the antitoxin in man.

I DAVIDSOHN

**PROPERTIES OF ANTIGENIC PREPARATIONS FROM BRUCELLA MELITENSIS** A. A. MILES and N. W. PIRIE, *Brit. J. Exper. Path.* **20** 109, 1939

Miles and Pirie describe the serologic behavior of the antigen of *Brucella melitensis* in its native state and after stepwise degradation by various agents. The relationship between the physical properties of a solution of the antigen and the character of the serum precipitate is discussed.

**ACTION OF PROTEOLYTIC ENZYMES ON THE ANTITOXINS AND PROTEINS IN IMMUNE SERUM** C. G. POPE, *Brit. J. Exper. Path.* **20** 132, 1939

Pope has reexamined the possibility of purifying antitoxic serum by digestion of inactive protein with proteolytic enzymes (mainly pepsin). Working with diphtheria antitoxic serum, he has determined the conditions necessary for maximum digestion of inactive protein and minimum loss of antitoxic activity. Samples of plasma from different horses show considerable variation in response to pepsin action, usually plasma from horses which have responded well to immunization give the best results. It is concluded that peptic digestion alone does not provide a suitable method for the purification of antitoxin since the degree of purification is rather low (up to four times) and the products of the breakdown of inactive protein are difficult to separate from the antitoxin.

CULTIVATION OF THE VIRUS OF AUJESZKY'S DISEASE ON THE CHORIOALLANTOIC  
MEMBRANE OF THE DEVELOPING EGG R E GLOVER, Brit J Exper Path  
20 150, 1939

No effective method has yet been devised for inducing satisfactory immunity against Aujeszky's virus disease of animals (pseudorabies). Glover has endeavored to adapt the virus to the developing egg in the hope that the infective agent would undergo mutation and serve as an immunizing agent. The virus has been successfully propagated on the egg. Some evidence of mutation has been obtained, but the virus is not yet sufficiently attenuated to permit employment of it for purposes of vaccination.

### Tumors

CEREBELLAR ASTROCYTOMA P C BUCY and W A GUSTAFSON, Am J Cancer  
35 327, 1939

Cerebellar astrocytomas occur for the most part in children. They are well circumscribed solid or cystic gliomas, which can usually be readily and successfully enucleated. They are composed predominantly of fibrillary and protoplasmic astrocytes in variable proportions, in association with a very small percentage of other adult and embryonic cells of the spongioblastic series. They contain no ganglion cells or nerve fibers other than those engulfed as a result of their invasion of the cerebellum and no neuroblasts. They not infrequently invade the subarachnoid space, and in such areas one may find glial bridges connecting the molecular layer of the cerebellum and the subarachnoid space. Degenerative changes involving the cells of the tumor, their processes and fibrillae and the blood vessels are common and are not infrequently misinterpreted by the unwary. The surrounding cerebellum shows the effects of compression and ischemia. Although it is possible that many cerebellar astrocytomas are congenital, arising from a developmental fault, this hypothesis remains unproved. There is no evidence to support the contention that these are congenital malformations rather than neoplasms. The small intraneoplastic cysts which develop are the result of liquefaction of the tumor tissue, but the extraneoplastic cysts, and perhaps the large intraneoplastic ones as well, appear to be formed by transudation. The original classification of these tumors into fibrillary and protoplasmic astrocytomas is accurate and valuable. More recent efforts at alteration of the classification or of its nomenclature are illogical, of little value and confusing.

FROM AUTHORS' SUMMARY

THE PRODUCTION OF CARCINOMA AND SARCOMA IN GUINEA-PIGS BY THOROTRAST  
[COLLOIDAL THORIUM DIOXIDE] L FOULDS, Am J Cancer 35 363, 1939

Four injections of 0.2 to 0.3 cc of colloidal thorium dioxide made into the base of a nipple produced tumors in 4 of 9 guinea pigs which survived until the earliest growth was detected. The average induction time was about thirty-seven months. The tumors comprised 1 carcinoma, 2 sarcomas and 1 fibrosarcoma, the carcinoma and the sarcomas were transplantable. The carcinoma has been transmitted through fifteen generations. The experiment demonstrated the production of a carcinoma other than a squamous carcinoma by the local action of a carcinogenic agent.

FROM AUTHOR'S SUMMARY

IMMUNITY TO SHOPE FIBROMA VIRUS J CLEMMESSEN, Am J Cancer 35 378,  
1939

Shope fibroma virus injected into rabbits treated by general roentgen irradiation produces results similar to those described by Andrewes and others in rabbits given injections of tar, namely, prolonged growth of the resultant fibromas, prolonged resorption and general fibromatosis, after intravenous inoculation. The development of immunity to repeated inoculations with this virus is delayed in roentgen irradiated rabbits. The effects of general roentgen irradiation on transplanted tumor and normal cells, on inoculated fibroma virus and artificial carcino-

genesis have their parallels in the effects of injections of tar and of trypan blue. It is suggested that all these procedures act through the reticuloendothelial system.

FROM AUTHOR'S SUMMARY

EFFECT OF OIL OF WINTERGREEN ON SPONTANEOUS TUMORS L. C. STRONG, *Am J Cancer* **35** 401, 1939

Extensive liquefaction of spontaneous tumors of the mammary glands in mice may be brought about by introducing heptylaldehyde into the organism through the diet. Liquefaction of spontaneous tumors may also be produced by injecting heptylaldehyde subcutaneously in areas remote from the neoplasm. Because of hemorrhage from the surface of the tumor and absorption of possible toxic agents from large or advanced growths, great caution should be employed in the use of heptylaldehyde or of any other agent that has a similar action on spontaneous tumors. The synergistic or tandem use of two or more chemicals may be the means of controlling spontaneous tumors by chemotherapy, at least in mice.

FROM AUTHOR'S SUMMARY

CONTORTED MITOSIS AND THE SUPERFICIAL PLASMAGEL LAYER W. H. LEWIS, *Am J Cancer* **35** 408, 1939

The assumption that cells have a superficial layer of gelated cytoplasm (plasmagel layer) which automatically exerts continuous contractile tension and that this layer undergoes various local and general changes in viscosity and thickness, with corresponding variations in its contractile tension, offers a key to one of the important factors concerned in changes of cell form, in cell locomotion and in cell division. The contorted mitoses observed in the division of the cells of spindle cell sarcoma C37 are explained by the development of changing contraction bands of the plasmagel layer which produce constrictions that result in marked distortions and lobulations of the dividing and young daughter cells.

FROM AUTHOR'S SUMMARY

EXPERIMENTAL ADENOMA OF THE PITUITARY I. H. PERRY and M. S. LOCKHEAD, *Am J Cancer* **35** 422, 1939

Three pituitary tumors developed in 131 female mice receiving prolonged treatment with estrone (theelin), none was present in 97 controls. Two of these mice also had inguinal implants of 1,2,5,6-dibenzanthracene.

FROM AUTHORS' SUMMARY

PULMONARY ADENOMATOSIS (JAGZIEKTE) IN SHEEP C. BONNE, *Am J Cancer* **35** 491, 1939

In the human lung a diffuse tumor-like condition, here described as carcinosis, may occasionally be found. Morphologically, this closely resembles certain pulmonary diseases of animals, of which jagziekte in sheep is the best known representative. In these diseases the alveolar cells and sometimes the bronchiolar cells are replaced by dark-staining cells in which mitoses are frequent. These cells line the alveolar sacs and often protrude into their lumens, forming papillomatous buds. There is practically no invasive growth, and metastases are absent. The possibility of a virus origin of these diseases is discussed. A detailed description is given of a case of general pulmonary carcinosis in a Chinese.

FROM AUTHOR'S SUMMARY

# Society Transactions

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## CHICAGO PATHOLOGICAL SOCIETY

S A LEVINSON, *President*

EDWIN F HIRSCH, *Secretary*

*Regular Monthly Meeting, Oct 9, 1939*

PRESIDENTIAL ADDRESS I THE HISTORY AND PROGRESS OF THE SCIENTIFIC  
WORK OF THE COOK COUNTY CORONER'S OFFICE SAMUEL A LEVINSON

The coroner's office in Cook County was established in 1864 Ernst Schmidt, the first coroner of Cook County and the first medical man to hold this position, was elected on the Republican ticket at this time

Schmidt, who was a liberal and an exponent in the defense of civil and political liberties, graduated from the University of Wurzburg in 1857 and arrived in Chicago in the same year During his student days in the University of Wurzburg he was active in the revolutionary movement in the year 1848-1849 There he later attended the lectures of Virchow and became his enthusiastic disciple and friend Schmidt and Virchow had a common bond in that both were interested in social and political reforms When Schmidt arrived in the United States, and later in Chicago, he carried on these reforms along the lines of a defender of civil rights and liberties, and among other activities he "stumped" the state of Illinois for Abraham Lincoln for President of the United States One outstanding example of the work performed by Schmidt was his active part in the defense of the "Haymarket rioters" in Chicago in 1887

Schmidt aided in the founding of the Humboldt College of Medicine in St Louis, and at the outbreak of the Civil War he joined the third Missouri brigade and was with this unit until illness forced him to retire On his return to Chicago in 1864 Schmidt was elected the first coroner of Cook County He was regarded as one of the first scientific men in medicine, as well as one who had received training in pathology under Rudolf Virchow Schmidt was coroner of Cook County for a little over a year, when he resigned because of his inability to cope with political influences in his office He was interested in making anatomic dissections and postmortem studies in the various cases of death referred to his office, but was unable to carry on the scientific work

In 1890 Henry Hertz was elected coroner of Cook County, and he appointed the first coroner's physician—Ludvig Hektoen

Hektoen's experiences as coroner's physician during this time are comparable to the experiences of most physicians who covered large territories with horse and buggy Hektoen's work included investigations of deaths due to natural causes, and he issued death certificates His duties also consisted of making autopsies at the Cook County Hospital for the coroner, as well as investigating causes of deaths and making autopsies in the various undertaking rooms in Chicago and Cook County There were no streetcars or elevated lines extending to the suburban areas, and the horse and buggy method of conveyance took considerable time Because of the large area that had to be covered, Hektoen could not always complete the work assigned each day Aside from duties as coroner's physician he was called on to testify in criminal cases, which took a great deal of time He was also professor of pathology at the College of Physicians and Surgeons Hektoen could not keep detailed records of all the autopsies performed during this time, but he did make use of the interesting pathologic material that presented

itself. There were no medical libraries available, the literature was scanty, and he was pressed for time to study in detail the material he wished to report. A review of the pathologic material presented before the Chicago Pathological Society at its beginning reveals an amazingly large number of studies made by Hektoen on gross as well as on histologic material which he had obtained as coroner's physician. More than forty articles were published during the time that he held this position.

Hektoen, as first coroner's physician, started a system which he called "investigation record," giving the facts as he found them at the autopsy table. He urged strongly that records of observations made at autopsy were of great importance to the coroner's office for their legal as well as scientific value.

Louis J. Mitchell, who had started his internship at the Cook County Hospital six months before Hektoen, was the second coroner's physician in Cook County and served for four years. One of the important autopsies performed by Mitchell, who had called Hektoen in as consultant, was that on the body of Mayor Carter Harrison, who had been assassinated by Prendergast.

When Peter M. Hoffman was elected coroner of Cook County in 1912, he organized an advisory committee to recommend competent candidates for coroner's physicians as well as to advise on other matters. E. R. LeCount was appointed coroner's physician and was stationed at the Cook County Hospital Morgue.

Hektoen recently wrote the following concerning LeCount:

"He set a high standard of necropsy and of necropsy records. In connection with his medicolegal work he developed a well organized system of volunteer assistantships. The duties were indeed arduous but the opportunities to learn were so attractive that there never was any lack of acceptable applicants. The work began between four and five o'clock in the morning so that it might be finished before the hour of the regular course in pathologic anatomy. So tireless was LeCount's industry, so fully had he taken himself in hand, that during these years he rose shortly before four o'clock. In the course of his medicolegal service he gathered a remarkable series of records of cases (99 volumes, fully indexed), which form a copious source of information concerning the pathologic anatomy of medicolegal conditions. He was engaged in the study of this material to the last, and in his room in the hospital he completed valuable analyses of cases of fractures of the skull and of gunshot wounds involving the chest and abdomen simultaneously.

"As the years passed he developed into an authority in practical pathology, clinical and medicolegal. His experience, the skill and thoroughness of his examinations, his wide knowledge, his refusal to come to any final decision in the absence of conclusive evidence, and the soundness of his judgments gave him the standing of a supreme judge in questions of diagnosis on a structural basis."

From 1923 to 1927 Oscar Wolff was coroner of Cook County and we can refer to this era only as the "four dark years."

As a result of Wolff's administration as coroner of Cook County, the law-abiding citizens of Chicago and Cook County made a protest against the method and conduct of the coroner and drafted Herman M. Bundesen as a candidate to oppose Wolff. Bundesen was elected by an overwhelming majority.

Bundesen assumed office in 1928 and resigned in 1931 to become health commissioner. During his tenure in office he introduced numerous reforms, reorganized his staff of pathologists and elevated the scientific division of the coroner's office to its present high level. He also appointed an advisory committee consisting of Ludvig Hektoen, J. P. Simonds, W. F. Petersen, Frank McJunkin and Louis Schmidt.

F. J. Walsh, the present coroner of Cook County, has continued along the lines recommended by his medical advisory committee and has at no time interfered with the scientific and professional work of his physicians and toxicologist. He has maintained his scientific staff at its present high level.

W. D. McNally was appointed first coroner's chemist, and Ralph Webster served for about one year. C. W. Muehlberger is the present coroner's toxicologist.

## II ANALYSIS OF THE WORK DONE BY THE SCIENTIFIC STAFF OF THE COOK COUNTY CORONER'S OFFICE

The percentage of all deaths occurring in Cook County which are investigated by the coroner's office varies from 12.73 to 21.63. This percentage is much higher than has heretofore been reported by various analysts. The total number of cases investigated by the coroner's office varied from 4,937 in the year 1909 to 9,011 in the year 1928.

The number of autopsies performed by coroner's physicians during the years 1931 to 1938 is also much higher than has previously been reported. The percentage of all deaths due to natural causes as well as deaths resulting from trauma or violence on which autopsies were done varied from 17.32 in 1932 to 26.4 in 1935. The percentage of deaths investigated by the coroner's office on which autopsies were done compares favorably with that of similar offices in the United States, and it certainly compares favorably with that in most of the hospitals of Chicago.

An analysis was made of the number of deaths resulting from homicide, suicide, poisoning and abortions. The highest level of deaths due to homicide was in 1929, during the prohibition era. When the Prohibition Act was repealed by Congress, in 1933, there was a sudden drop in the number of homicides.

Deaths due to suicide had two peaks, one in 1915, during the World War, and another in 1929-1930, during the economic crash. The economic crash was not the sole factor in the increase in the number of suicides in 1929-1930, and a more detailed analysis of this will be reported elsewhere.

A similar analysis was made of the number of deaths due to poisons as well as of the number due to abortion.

In 1929 the firearms investigation bureau was established in Chicago. The coroner's office has a ballistics unit which is an integral part of this office. The firearms identification bureau examined as many as 1,431 guns in the year 1932. In 1930 647 bodies dead from bullet wounds were submitted to autopsy, and 1,131 bullets were recovered and given ballistics examination. The toxicologic department, which is an integral part of the coroner's office, examines not only material submitted from the bodies dead from or suspected of being dead from poisoning but medicines and other chemicals found on the dead body or in the vicinity of the dead body. For example, in 1929 there were 994 bodies dead from or suspected of being dead from poisoning, and 1,277 examinations were made for poisons and on material submitted by the Cook County Purchasing Department.

The material obtained at coroner's autopsies differs in many respects from that obtained at "permission" autopsies. It includes specimens representative of unexpected or sudden deaths, as well as deaths from poisoning, drowning, electrocution, abortion, bullet wounds, industrial and transportation accidents, etc. The material is utilized for teaching purposes as well as for exhibits.

A course for the teaching of legal medicine in medical schools has been organized in the past few years.

The scientific work performed by the coroner's office should form a nucleus for the establishment of an institute of legal medicine in Cook County. The crime detection laboratory, the department of police administration, the institute for juvenile research and the psychiatric institute, and the firearms identification bureau are all services which are directly or indirectly extensions of the pathologic and toxicologic departments of the coroner's office. These can be utilized in aiding the courts and prosecution as well as the defense in the conduct of legal cases. Although they function independently, they aid one another in a cooperative way. If this loose integration could be organized into a close corporation and associated with the legal group, a true type of medicolegal institute would be approached which would be of great service to the citizens of our community.

# Occurrence and Significance of Congenital Malignant Neoplasms H GIDEON WELLS

Human cancer when produced by known agencies (e g, roentgen rays, radium, or chemicals as in dye workers' cancer) has been found usually to require seven to fifteen years for its development. Experimental study of cancerogenesis has shown that such a long total period of development is not necessary but that what is necessary is a comparable part of the normal life span. Hence experimental cancer appears quicker in mice than in rabbits, in rabbits than in dogs, in dogs than in man. In striking contrast to this general principle is the fact that there is a group of tumors that usually appear early in life or not at all, outstanding of which are the neuroblastoma of the retina, the neuroblastoma of the adrenal gland and the malignant nephroma. A study of cases of these tumors shows that in a considerable proportion they are already present as malignant neoplasms at the time of birth. In our laboratories, in about 3,000 necropsies on fetuses born dead or on infants dying shortly after birth there were observed 3 instances of congenital adrenal neuroblastoma and 1 instance of neuroblastomatous involvement of much of the sympathetic nervous tissue.

## *Summary of Recorded Congenitally Malignant Tumors (Not Including Retinal Tumors)*

	Accepted	Probable	Possible	Total
Malignant renal tumor	5	11	?	16
Malignant adrenal neuroblastoma	17	15	21	53
Malignant extra adrenal neuroblastoma	6	4	?	10
Congenital sarcoma	33	29	33	115
Teratoma with malignant character at birth	1	2	?	3
Tumors of undetermined nature	3	1	7	11
Carcinoma of the liver	0	1	9	10
Hemangioendothelioma malignum of the liver	?	?	15	15
Tumor of the liver of undetermined character	?	?	4	4
Carcinoma, excluding liver	0	0	5	5
Cerebral glioma	1	1	2	4
Malignant endothelioma, excluding liver	?	?	5	5
Melanoma malignum	0	2	2	4
	66	66	123	255

In the hope that a study of cases of malignant growth present at birth might offer some suggestions as to the meaning of this deviation from the usual requirements for malignant neoplasia, a review of the literature has been made, and many misinterpretations have been corrected. The results are summarized in the accompanying table.

There have been no congenital carcinomas of the types commonly seen in adults which were indisputably established to be such, although a few lesions reported as congenital carcinoma of the liver or thymus may be genuine. On the other hand, there are not a few unquestionably congenital sarcomas which seem to differ in no noteworthy respect from the sarcomas of adults. Since it requires about the same length of time to produce experimental sarcomas and carcinomas, the explanation for this difference is not clear.

While a large proportion of neurogenic malignant tumors derived from the tissues of the sympathetic nervous system and from the retina, when present, are present at birth, congenital neoplasms of the brain and peripheral nerves are extremely rare. Evidence is found that congenital maldevelopments of nerve tissues occur frequently in brain, retina and adrenal, and these apparently may disappear, develop into malignant tumors, develop into benign tumors or even become malignant and then change in whole or in part into nonmalignant tissues.

In striking contrast to retinoblastoma in which there is a marked hereditary factor, the other types of tumor tend to be congenital or, if they appear in early infancy, show no familial or hereditary influences.

Placental tumors, occurring as they do in fetal tissues, may not be an exception to the rule that from one fifth to one tenth of the life cycle of an organism ordinarily is required to produce a malignant growth, since the placenta is an organism the entire life cycle of which terminates in senility in about six months

Some cases of true congenital leukemia have been reported, but not a single instance has been found in which the leukemia was transmitted from the mother to the fetus, although there have been a few instances of metastasis of maternal cancer to the fetus

The significance of congenital malignant growth is discussed in the light of the fact that ordinarily a large part of the life cycle is required to produce any type of malignant tumor

Presumably the malignant growths which appear chiefly before or soon after birth depend on some other mechanism or principle than do the ordinary malignant growths that require so long for their production Cramer has directed attention to the fact that the retinoblastoma, which appears almost exclusively in infants, and the chorionepithelioma arise from tissues that have a type of metabolism resembling that of tumors Besides the retinoblastoma, the neuroblastoma and the nephroblastoma are also tumors of the first few years of life, and in recent years it has been shown that in the brain and the kidney medulla glycolysis is high Thus these tissues also have a metabolism resembling that of the embryo and of tumors, and this may have some connection with the occurrence of these tumors in the early years of life

#### DISCUSSION

S R ROSENTHAL I have seen a number of swine with Wilms' tumor of the kidney in a benign form The tumor usually occupies one pole of the kidney It sometimes becomes very large but never metastasizes Histologically, there are rudimentary tubules and glomeruli in a matrix of cellular connective tissue

#### TUMORS OF THE SYMPATHETIC NERVOUS SYSTEM EDITH L POTTER

Neuroblastoma and ganglioneuroma are tumors composed of cells representing different degrees in the development of primitive tissue arising originally from the neural crest of the embryo The structure varies from that of tumors which are composed entirely of completely undifferentiated cells (sympathogonia), moderately differentiated cells (sympathoblasts) or completely differentiated cells (ganglion cells) to that of tumors in which there is a mixture of all elements Schwannoma has been found in association with chromaffin tumors but only rarely in association with neuroblastoma or ganglioneuroma

The present case is that of an infant born dead and two months prematurely, in whom multiple tumors were present The entire chain of paravertebral sympathetic ganglions was transformed into a continuous mass of tumor tissue, 1 to 15 cm in diameter Fused with the inferior surface of each adrenal were lobulated, circumscribed tumors, each 5 by 5 by 6 cm Connecting these across the midline was a similar tumor of approximately equal size The wall of the urinary bladder was firmer, more fibrous tumor tissue, 3 cm in maximum thickness It extended posteriorly and encircled the rectum, the fibers ending at the anus Numerous small encapsulated masses of tumor tissue were in the posterior part of the abdominal cavity There was extreme hypertrophy of all nerves in the body, particularly of those of sympathetic origin Multiple minute tumor nodules were present throughout the liver

Microscopically, all of the tumors had a similarity in structure and were composed of sympathogonia, sympathoblasts, ganglion cells and nerve fibers, with irregular regions of hemorrhage, calcification and necrosis Sympathogonia formed the main portion of the adrenal tumors, ganglion cells and fibers composed the greater part of the ganglion tumors, but all types formed a diffuse mixture in all tumors

The tumor of the bladder differed from the tumors in other locations It had large masses of tissue composed of elongated nuclei and fibrillar material separated



into bundles of collagen fibers. In one region groups of immature ganglion cells were present, but most of the tumor consisted only of Schwann cells and fibers, characteristic of schwannoma. The hypertrophy of the nerves was due to an increase in Schwann cells accompanied by local excessive proliferation of acellular fibrils.

This case exemplifies the interrelationship of neuroblastoma, ganglioneuroma and schwannoma. The tumor is due not to activation of misplaced rests of embryonic tissue but to some condition affecting the entire sympathetic nervous system, stimulating generalized neoplastic growths.

INTRACAPILLARY MICROSCOPIC METASTATIC MAMMARY GLAND CARCINOMA OF THE LUNGS AND OTHER VISCERA. LESLIE R. GRAMIS

This report was published in full in the December issue of the ARCHIVES, page 865.

## Book Reviews

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**Medical Jurisprudence and Toxicology** William D McNally, A B, M D, Assistant Professor of Medicine and Lecturer in Toxicology, Rush Medical College, University of Chicago Pp 386 Price \$3.75 Philadelphia W B Saunders Company, 1939

The book is divided into two sections part 1, "Medical Jurisprudence," 69 pages, part 2, "Toxicology," 259 pages Part 1, which is supposedly written primarily for the physician and the medical student, is entirely inadequate and the treatment rather superficial About a ninth of the book has been allotted to the topic of medical jurisprudence, and essential phases have been omitted, or, if mentioned, have not been described in sufficient detail, for example, the matter of the signs of death, an important medicolegal problem, has been disposed of in 3 pages, sudden and unexpected deaths from natural causes, which on the average constitute more than 50 per cent of the total number of deaths that come to the attention of the coroner or the medical examiner, have been allotted but 2 pages, injuries, burns, abortions, infanticides and insanity are lumped together and rather inadequately treated in the space of 25 pages, bullet wounds, stab wounds and rape are mentioned very briefly, while, in contrast, ballistics, a matter primarily of concern to the police, has been given 9 pages! A further criticism of this section is that numerous careless and misleading statements are made which will raise doubts in the mind of any discriminating reader One might mention particularly the section on blood grouping, especially the paternity table on page 71, in which 12 errors have been made Any one using this table would be grossly misled

In the second part of the book, that on toxicology, the symptoms of poisoning and the treatment of various types of poisoning are given in a concise form and are well presented However, the discussion of the detection and the quantitative determination of the poisons that may be present in tissues is fragmentary and indefinite In the description of the methods abrupt transitions and poor sequences leave much to be desired An inexperienced analyst attempting to carry out an analysis of organs for poisons by following the directions in the text could not hope for much success, an experienced analyst would not be apt to use the book

Many important laboratory procedures are scantily treated, only general principles being outlined Some of the more recent and specific tests of importance have not been mentioned Methods of isolation and purification of toxic principles, which must precede the application of identification tests, have in the case of several of the poisons been totally ignored Crystal tests for several of the alkaloids do not appear Concerning the chemical procedures many ambiguous and erroneous statements are made

One realizes that it is impossible to confine in a volume of this size detailed descriptions of all drugs and chemicals that may enter into suicides, homicides and accidental poisonings, but one does feel that if any tests are given they should be so stated that a person with the necessary fundamental training can carry them out successfully This the book does not do, and therefore it cannot be considered as satisfactory to serve as a textbook on toxicology

## Books Received

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COMMUNICATIONS DU LABORATOIRE BACTÉRIOLOGIQUE DE L'ÉTAT SUÉDOIS  
Volume XII, 1937 Paper Various pagination Uppsala Almqvist & Wiksells  
Boktryckeri-AB, 1939

REPORT OF THE PRESIDENT AND OF THE TREASURER FOR THE YEAR ENDED  
SEPTEMBER 30, 1939, CARNEGIE CORPORATION OF NEW YORK Paper Pp 167  
New York Carnegie Corporation of New York, 1939

PROCTOSCOPIC EXAMINATION AND DIAGNOSIS AND TREATMENT OF DIARRHEA  
M H Streicher, M S, M D, University of Illinois College of Medicine, Chicago  
Cloth Pp 149, with 39 illustrations Price \$3 Springfield, Ill Charles C  
Thomas, Publisher, 1940

REPORTS ON BIOLOGICAL STANDARDS—V VARIABLES AFFECTING THE ESTI-  
MATION OF ANDROGENIC AND OESTROGENIC ACTIVITY C W Emmens Medical  
Research Council, Special Report Series, no 234 Paper Pp 71 Price 40  
cents, 1 shilling, three pence New York British Library of Information  
London His Majesty's Stationery Office, 1939

A SYMPOSIUM ON THE BLOOD AND BLOOD-FORMING ORGANS E Meulengracht,  
Cecil James Watson, C P Rhoads, Clark W Heath, George R Minot, Louis  
K Diamond, Russell L Haden, J Furth, Claude E Forkner, E B Krumbhaar,  
Charles A Doan, Hal Downey, Paul Reznikoff, Edwin E Osgood and Harry  
Eagle Cloth Pp 264, illustrated Price \$3.50 Madison The University of  
Wisconsin Press, 1939

THE RISE OF EMBRYOLOGY Arthur William Meyer, Professor of Anatomy,  
Emeritus, Stanford University Cloth Pp 367, with 97 illustrations Price \$6  
Stanford University, Calif Stanford University Press, 1939

CLINIQUE ET PHYSIOPATHOLOGIE DES MALADIES COELIAQUES Robert Dubois,  
Agrége de l'enseignement secondaire, Medecin-adjoint de la Clinique infantile de  
l'Université de Bruxelles Preface du professeur E J Bigwood Paper Pp  
350, with 14 illustrations Price 80 francs Paris Masson & Cie, 1939

## INDUCTION OF GASTRIC TUMORS IN STRAIN A MICE BY METHYLCHOLANTHRENE

HAROLD L STEWART, MD

Pathologist, United States Public Health Service

BFTHESDA, MD

Reports of the production of tumors of the stomach in experimental animals are so uncommon that their number is out of all proportion to the importance and frequency of this lesion as it occurs in man

Considering the large number of animals that have been subjected to experimental tarring or painting with pure carcinogenic chemicals, the development of gastric neoplasms has been extremely rare. The Tworts<sup>1</sup> stated that among 60,000 mice painted with tar small papillomas of the stomach were observed only occasionally. After examining the stomachs of several hundred tarred mice, Bonne<sup>2</sup> found papillomas in a few animals which had survived two hundred days and in 1 animal a squamous cell carcinoma of the forestomach. Shabad<sup>3</sup> also reported the production of a benign papillomatous tumor in the forestomach of a mouse in a group that had received tar by rectal injection. Twort and Bottomley<sup>4</sup> reported a squamous cell epithelioma in the forestomach of a mouse whose skin had been painted for twenty weeks with chrysene ammonium and sodium sulfonate, they seem to have believed it a spontaneous tumor, since in 12,000 mice with induced cutaneous tumors this was the only tumor of a mucous membrane. On the other hand, the belief has been expressed that such neoplasms of the alimentary canal may result from ingestion of the carcinogenic chemical by the animal's licking the painted area or consuming contaminated food. However, this would not explain the gastric papilloma and carcinoma in female mice painted with 1,2,5,6-dibenzanthracene combined with theelin, reported by Perry and Ginzton,<sup>5</sup> since tumors of the stomach have not been found to be associated with uncomplicated painting of the skin with 1,2,5,6-dibenzanthracene in other laboratories where this procedure is common practice.

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From National Cancer Institute, United States Public Health Service

1 Twort, J. M., and Twort, C. C. *J. Path. & Bact.* **35**: 219, 1932

2 Bonne, C. *Ztschr. f. Krebsforsch.* **25**: 1, 1927

3 Shabad, L. M. *Vestnik rentgen i radiol.* **6**: 223, 1928

4 Twort, C. C., and Bottomley, A. C. *Lancet* **2**: 776, 1932

5 Perry, I. H., and Ginzton, L. L. *Am. J. Cancer* **29**: 680, 1937

The injection of carcinogenic agents into the wall of the stomach and the oral administration of these substances in a deliberate attempt to provoke gastric tumors have yielded positive results in some cases and negative results in others. Uehlinger and Schurch<sup>6</sup> introduced a mixture of 0.005 mg of mesothorium in petrolatum into the gastric wall in 8 rabbits, 2 of which died twenty-one months and twenty-nine months later with sarcoma of the stomach. Ilfeld<sup>7</sup> inserted pellets of benzpyrene under the gastric mucosa in 6 ferrets and pellets of 1,2,5,6-dibenzanthracene into the gastric wall of a dog, with negative results after a year. Fibiger<sup>8</sup> found that carcinoma of the stomach developed in mice as well as in rats, when they were infected with spiroptera. However, this work has recently failed of confirmation by Cramer.<sup>9</sup> There is a case mentioned by Creighton<sup>10</sup> as having been reported by Twort in which a cancer of the pylorus of a mouse apparently resulted from injury produced by a piece of swallowed glass. A papillary carcinoma in the forestomach of a male mouse was reported by Mercier and Gosselin<sup>11</sup> as following an intraperitoneal injection of tar in olive oil. Voronoff and Alexandrescu<sup>12</sup> administered a mixture of tar, hydrous wool fat, aniline oil and toluylenediamine by mouth to 10 rats and observed the development of carcinoma of the stomach in 1. Tani<sup>13</sup> found tumor-like changes resembling carcinomatous areas in the wall of the forestomach in mice receiving tar by mouth. Roffo<sup>14</sup> found that ingestion of fats that had been oxidized by heating and added to the ordinary food (bread and milk) of the white rat regularly provoked in these animals ulcer, papilloma and adenocarcinoma of the cardiac and pyloric chambers of the stomach during an experimental period of two years. He ascribed the cancer-producing role of these fats to the formation of oxysterol. He<sup>15</sup> also reported that in the rat ingestion of a diet containing cholesterol irradiated by the sun or with ultraviolet rays results in gastric adenocarcinoma. Waterman<sup>16</sup> found that cholesterol oleate administered orally alone, without any addition of known carcinogenic substance, produced malignant changes in the stomach. In 4 animals infiltrating papilloma with hyperkeratosis was produced and

6 Uehlinger, E., and Schurch, O. *Deutsche Ztschr. f. Chir.* **251** 12, 1938

7 Ilfeld, F. W. *Am. J. Cancer* **26** 743, 1936

8 Fibiger, J. *J. Cancer Research* **4** 367, 1919

9 Cramer, W. *Am. J. Cancer* **31** 537, 1937

10 Creighton, C. *Some Conclusions on Cancer*, London, Williams & Norgate Ltd., 1920, p. 120.

11 Mercier, L., and Gosselin, L. *Compt. rend. Soc. de biol.* **113** 669, 1933

12 Voronoff, S., and Alexandrescu, G. *Neoplasmes* **8** 129, 1929

13 Tani, I. *Tr. Jap. Path. Soc.* **21** 715, 1931

14 Roffo, A. H. *Bol. Inst. de med. exper. para el estudio y tratamiento del cancer* **48** 407, 1938

15 Roffo, A. H. *Bol. Inst. de med. exper. para el estudio y tratamiento del cancer* **46** 589, 1938

in 1 true adenocarcinoma of the stomach. Waterman<sup>16</sup> also fed a 0.4 per cent solution of benzpyrene in lard to 6 mice, several milligrams being consumed daily by each animal. In 5 animals squamous cell carcinoma of the stomach developed in from one hundred and twelve to three hundred and thirty-six days, and in 4 of these there were metastases to the lymph nodes, peritoneum, liver, spleen or lung. Van Prohaska, Brunschwig and Wilson<sup>17</sup> found that, of 48 mice receiving methylcholanthrene orally and observed for periods ranging up to one hundred and eighty-six days, 2 presented benign squamous epithelial papilloma of the fundus of the stomach. There have been a number of reports of negative attempts to produce cancer of the stomach by oral administration of carcinogenic hydrocarbons.<sup>18</sup> The experiments reported by Kinoshita<sup>19</sup> on the development of gastric tumors in rats receiving butter yellow, tetramethyldiaminobenzophenone and aminoazobenzene orally have not been sufficiently controlled to rule out the possibility of vitamin A deficiency as an etiologic factor in the production of the lesions described by him.

#### EXPERIMENTAL PROCEDURE

Thirty strain A male mice aged 3 months were employed in this experiment. The mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. They were maintained on a diet of dog chow<sup>20</sup> exclusively except for the first few days after being received in this laboratory, during which time the diet was supplemented with bread and milk. Tap water was allowed freely at all times. When a mouse was to be given an injection it was anesthetized with pentobarbital sodium, and the stomach was exposed through a midline abdominal incision. The stomach of the mouse is composed of two chambers, sharply demarcated from each other on both the internal and the external surface of the viscus. The left, or cardiac, chamber is lined by squamous epithelium directly continuous with that of the esophagus. The pyloric chamber, which is continuous with the cardiac chamber, is lined by glandular epithelium, similar to that of other mammals, and terminates in the duodenum. A solution of methylcholanthrene in liquid petrolatum was injected into the anterior wall of either the cardiac or pyloric chamber or both. The solution contained 10 mg. of methylcholanthrene per cubic centimeter of liquid petrolatum and was injected in the dose of 0.03 to 0.05 cc. The solution became clear when heated to 40 C., at which temperature it was

16 Waterman, N. *Acta cancerol.* **2** 375, 1936.

17 van Prohaska, J., Brunschwig, A., and Wilson, H. *Arch. Surg.* **38** 328, 1939.

18 (a) Remhard, M. C., and Candee, C. F. *Am. J. Cancer* **26** 552, 1936. (b) Oberling, C., Sannic, C., Guerin, M., and Guerin, P. *Bull. Assoc. franç. p. l'étude du cancer* **25** 156, 1936. (c) Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Hieger, I., Kennaway, E. L., and Mayneord, W. V. *Am. J. Cancer* **29** 219, 1937. (d) Lorenz, E., and Stewart, H. L. Unpublished data. (e) Waterman<sup>16</sup>. (f) van Prohaska and others<sup>17</sup>.

19 Kinoshita, R. *Tr. Jap. Path. Soc.* **27** 665, 1937.

20 According to the Purina Mills Company, the chow contains the following ingredients: protein, 20 per cent, fat, 3 per cent, carbohydrate 56 per cent, ash, 6 per cent, and water, 15 per cent, with vitamins A and G added.

injected The clinical course of most of the animals remained satisfactory throughout the experiment In some animals palpable inflammatory masses developed in the upper part of the abdomen over the stomach The survival periods were as follows 1, 1, 7, 6, 3, 4, 2 and 3 mice lived for six, eight, ten, twelve, thirteen, fourteen, sixteen and seventeen months, respectively, 3 died postoperatively All the mice were examined post mortem except 1 mouse which was partially eaten by its cage mates

#### OBSERVATIONS

In 4 animals squamous cell carcinoma of the stomach developed, and in 4 squamous papilloma of the stomach The 4 mice with carcinoma of the stomach were submitted to autopsy ten, fourteen, fourteen and seventeen months, respectively, after the injection of the carcinogen, the 4 mice with papilloma of the stomach, eleven, thirteen, sixteen and sixteen months after treatment respectively In addition to the tumors the following lesions were noted in different animals at autopsy hyperplasia of the gastric mucous membrane chronic peritonitis with adhesions in the upper part of the abdomen multiple adenomatous tumors of the lung, chronic nephritis lymphoma, abscess of the liver, spleen and prostate gland, cirrhosis, amyloidosis chronic pancreatitis, pyloric obstruction, hemorrhage into the colon and stomach and ulcerative dermatitis

No tumors developed in 13 control mice with pieces of untreated yarn soaked in laid or in liquid petrolatum or with plain cotton thread stitched into the wall of the stomach

*Papilloma*—There were 4 cases of papilloma of the stomach Two of the tumors were solitary small gray nodules, 4 mm and 6 mm in diameter, respectively, each projecting into the lumen of the cardiac chamber of the stomach (fig 1A) The other 2 lesions were small and scarlike, and involved the mucous surface of the stomach The latter tumors were indistinguishable grossly from the scarring and thickening of the gastric wall which occurred occasionally as a result of inflammatory changes evoked by the presence of the hydrocarbon

Microscopic examination revealed that in 3 of the cases the lesion was located at the junction of the cardiac and pyloric chambers, while in the other case it was confined to the cardiac chamber Structurally each tumor consisted of a central stalk of connective tissue covered with squamous epithelium In 3 cases the stalk supporting the papilloma was broad and in the other case narrow and high The surfaces of the flat lesions were studded with spinelike projecting papillae The covering cells were fairly regular, well differentiated stratified squamous epithelium Mitotic figures were infrequent in 3 cases, and in the fourth case from two to four were observed in several single high power fields The epithelial covering of the tumor merged abruptly with the mucous membrane of the stomach on either side of the lesion, without any marked hyperplasia of the cells at the zone of transition but with slight hyperkeratosis in this region in 1 case The connective tissue

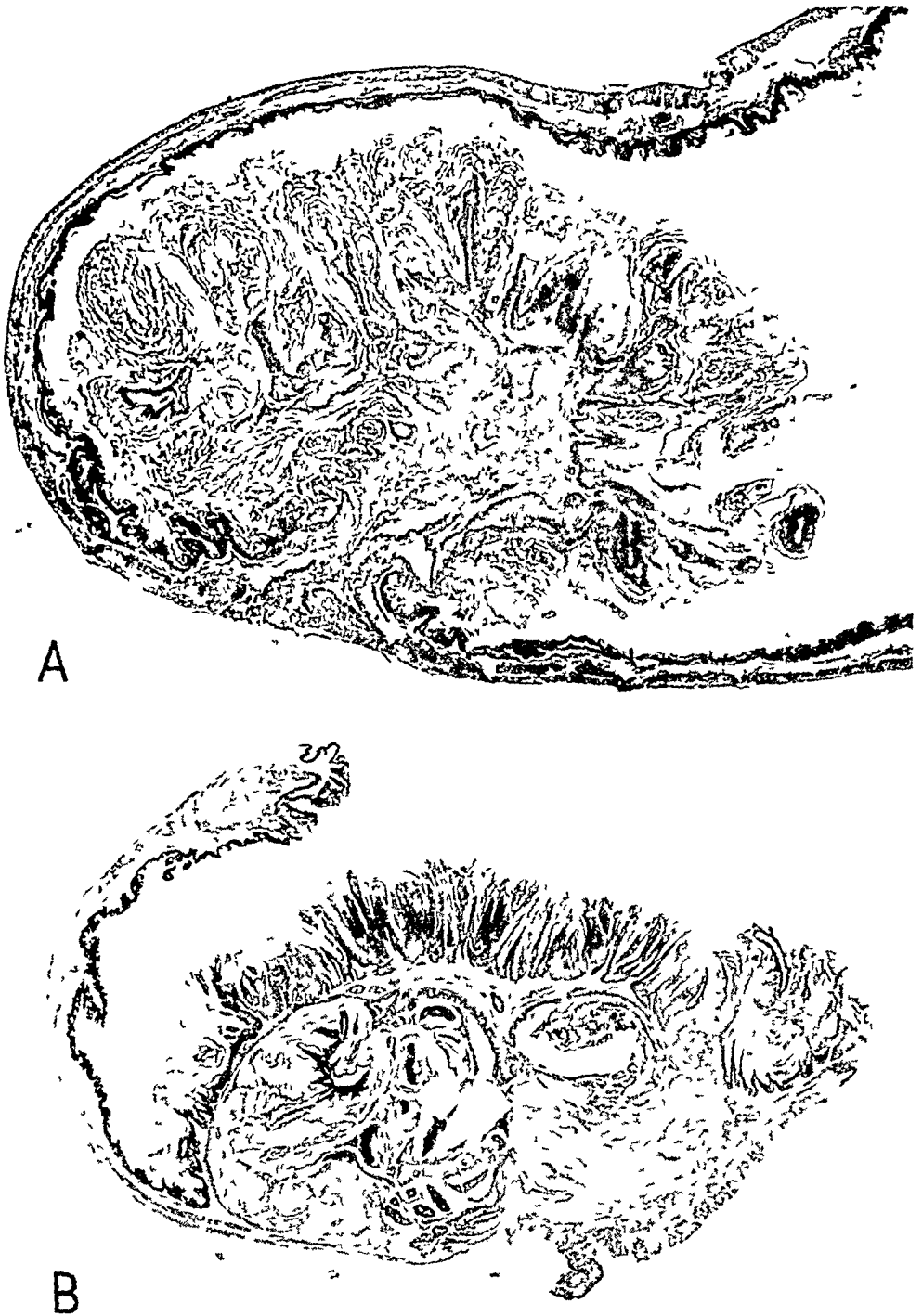


Fig 1—*A*, squamous papilloma of the cardiac chamber of the mouse stomach produced by injecting a solution of methylcholanthrene in liquid petrolatum into the wall of the stomach sixteen months previously,  $\times 25$  *B*, portion of the cardiac chamber of the stomach of a mouse, showing papillary squamous cell carcinoma,  $\times 10$  The mouse had received an injection of methylcholanthrene in liquid petrolatum into the wall of the stomach seventeen months previously



stroma was composed of adult fibroblasts with a few well formed blood vessels and in 1 case numerous inflammatory cells. The smooth line of demarcation between the epithelium and the underlying stroma, which is characteristic of a papilloma, was not always maintained. On the contrary, there was a tendency in all specimens for masses and strands of squamous cells to penetrate, in limited numbers, the underlying stroma, becoming apparently separated from the surface cells. In 3 cases this cellular infiltration did not go beyond the basement membrane of the gastric mucosa. In 1 case there was a small epithelium-lined cyst in the submucosa, adjacent to the muscular wall of the stomach.

*Carcinoma*—There were 4 cases of carcinoma of the stomach. The lesions measured from 5 to 9 mm in diameter. On cross section each consisted of a round gray nodule elevated 5 mm or so above the surface level of the mucous membrane of the cardiac chamber (fig 1 *B*) and all but 1 involved the anterior wall of the viscus. The latter was situated on the posterior inferior margin some little distance from the point of injection. Three lesions had finely granular surfaces, the fourth was a crater-like ulcer, covered with clotted blood and the lumen of the viscus was filled with blood. In 2 cases the tumor on the mucous surface of the stomach was continuous with a rounded umbilicated lesion on the peritoneal surface. The surrounding gastric wall was puckered and retracted on all sides toward the peritoneal extension of the tumor. In 1 case a portion of the liver adherent to the serosa of the stomach opposite the tumor contained a small abscess.

Microscopically the malignant tumors were papillary squamous cell carcinoma, showing extensive keratinization of the covering epithelium. Each was located in the cardiac chamber. The gastric mucous membrane at its junction with the lesion was hyperplastic and hyperkeratotic. One tumor was ulcerated with the base of the ulcer situated at the level of the circular musculature. The lumen of the stomach in this case was filled with blood. Every lesion exhibited frank infiltration of tumor cells into all the coats of the viscus. The infiltrating epithelium was composed of basal cells, prickle cells and flat squamous cells which were atypical in size, shape and staining and showed numerous mitotic figures (fig 2 *A*). The centers of the cellular masses frequently contained concentric layers of keratin, but in 1 tumor some of them were empty, suggesting an alveolar structure with a squamous lining. In 1 case there were nests of epithelium within thin-walled vessels either lymph vessels or blood vessels situated between the two muscular layers of the gastric wall (fig 2 *B*). The stroma of all the tumors was infiltrated with inflammatory cells, which were especially numerous along the course of the permeating tumor cells. In the case in which the liver was abscessed and adherent to the wall of the stomach opposite the



Fig 2—*A*, an area of the tumor illustrated in figure 1 *B*,  $\times 140$ . In this field there are masses and cords of atypical squamous epithelium infiltrating the wall of the stomach. *B*, the muscular wall of the gastric tumor shown in figure 1 *B* and figure 2 *A*,  $\times 400$ . The peritoneal surface is visible in the lower portion of the illustration. Between the muscle coats are two isolated masses of tumor cells within thin-walled vessels.

carcinoma there were masses of keratin in the center of the abscess. In 2 cases small nodules composed of tumor cells were adherent to the external surface of the peritoneum.

In 1 case bits of tissue from the squamous cell carcinoma of the stomach were transplanted into 6 male strain A mice, the transplants were inserted subcutaneously in the axilla in 3 animals and into the peritoneal cavity in 3 animals. Three weeks later 1 mouse with an axillary transplant and 3 mice with intraperitoneal transplants showed large tumors at the sites of inoculation. Transplants were then made from these inoculated tumors into corresponding locations in 6 strain A mice, and large tumors developed in 2 of these within one month. All the tumors that developed from the transplants were squamous cell carcinoma morphologically identical with the original tumor of the stomach from which they were obtained.

#### COMMENT

The results of this experiment seem to indicate that the mucous membrane of the stomach of the mouse, although susceptible to experimental induction of tumors, is relatively resistant to the carcinogenic action of methylcholanthrene as compared with other body tissues similarly treated on which data are available for comparison. In only 8 of the 30 mice given injections in this experiment did tumors of the stomach develop. It was not possible to determine accurately the latent period of tumor development, because the stomach was not inspected directly and abdominal palpation was of direct aid in determining the onset of tumor. However, a rough estimate of the time required for the lesions to attain the proportions noted at the postmortem examination may be gained from a consideration of the period which elapsed between the injection of the agent and the autopsy on the animal. This period averaged fourteen months both for papilloma and for carcinoma. This is considerably longer than the average time required for bulkier cutaneous or subcutaneous tumors to develop in mice of this strain treated with equivalent doses of methylcholanthrene. Additional evidence indicating the relatively greater resistance of the gastric epithelium of the mouse to induction of tumors was obtained in another experiment in which pieces of yarn soaked in a 5 per cent solution of methylcholanthrene in lard were stitched into the wall of the stomach in 14 C<sub>3</sub>H mice, in none of which did gastric tumors develop although 7 animals survived the experimental procedure by ten to seventeen months. In the present experiment, although the solution of methylcholanthrene in liquid petrolatum was injected into the wall of both the cardiac and the glandular chamber, the tumor arose only in the cardiac chamber, and in each instance it was of the squamous cell type thus

indicating still greater resistance on the part of the glandular mucous membrane of the stomach to the carcinogenic effects of methylcholanthrene as compared with the mucosa of the forestomach.

The negative results from many attempts to produce gastric tumors by feeding carcinogenic hydrocarbons has been thought to depend on the total insusceptibility of the gastric epithelium to the action of these agents or on the lack of absorption or on a chemical change occurring as a result of digestion. However, certain findings show that the resistance to the induction of tumors of the gastrointestinal tract by oral administration of 1, 2, 5, 6-dibenzanthracene to mice is not due to lack of absorption or chemical alteration. Lorenz and Stewart<sup>18a</sup> showed that pulmonary tumors develop in mice ingesting 1, 2, 5, 6-dibenzanthracene, indicating that this agent is absorbed in sufficient amounts to exert a carcinogenic action on lung tissue, furthermore, the hydrocarbon was found unchanged, in large part at least, in the alimentary canal up to the level of the ileocecal valve. That the gastric mucous membrane is not totally insusceptible to the carcinogenic action of methylcholanthrene and benzpyrene when these substances are administered orally is proved by the positive results obtained by Waterman<sup>16</sup> and van Prohaska, Brunschwig and Wilson.<sup>17</sup> The present experiment also shows that the resistance of the gastric mucous membrane of the forestomach to the induction of tumors is only a relative matter.

From the morphologic description of the lesions obtained in this experiment, it is apparent that the division of the tumors into papilloma and carcinoma was an arbitrary one for the purpose of classification. This division was made on the basis of the presence or absence of invasion of the deep muscular coats of the stomach by tumor cells. Such invasion was present in the lesions diagnosed as carcinoma, and it was absent in the specimens of papilloma. However, in the specimens of papilloma the smooth line of demarcation between the surface epithelium and the underlying stroma was not always maintained. Instead all these specimens exhibited to some extent excessive growth of epithelium with extension of tumor cells through this barrier into the underlying stroma in the form of masses and strands of cells apparently detached from the surface epithelium. In 1 case there was a mass of atypical epithelium below the basement membrane, in the submucosa. These findings suggest that under the conditions of this experiment transition stages may occur between papilloma of the stomach and carcinoma similar to the development of squamous cell epithelioma from benign warts of the skin on an area of the epidermis subjected to applications of a carcinogenic agent.

Of the attempts to produce experimental tumor of the stomach by injection of a carcinogenic agent into the wall of the stomach as reviewed in the introduction to this paper there are two which have a bearing

on the present experiments. Ilfeld<sup>21</sup> inserted pellets of benzpyrene under the gastric mucosa of 6 ferrets and 1, 2, 5, 6-dibenzanthracene pellets into the gastric wall of a dog with negative results after a year. Nothing is known at present regarding the latent period of induction of tumor in the ferret but a recent study by Passey<sup>22</sup> has shown that in the dog it requires several years to evoke tumors of the skin by painting with a carcinogenic tar. It therefore seems that the period of observation in Ilfeld's experiment might not have been long enough for positive results to be obtained. Uehlinger and Schurich<sup>23</sup> obtained sarcoma of the stomach in 2 of 8 rabbits following implantation of a mixture of mesothorium in petrolatum into the stomach wall. It is interesting that in the present experiment, although the carcinogen was likewise injected into the muscular and connective tissue of the wall of the stomach the tumors which developed were epithelial in type.

Consideration has been given the possibility that the gastric tumors obtained in the present experiment were spontaneous in origin. This possibility is unlikely, for spontaneous carcinoma of the stomach in mice is extremely rare. Wells, Slye and Holmes<sup>22</sup> reported that in 142,000 mice of the Slye stock, all dying of natural causes and most of them of cancer age without experimental manipulation, only 15 were found to have a primary malignant neoplasm of the stomach, 8 having squamous cell carcinoma of the cardia, 3 adenocarcinoma of the pylorus, 3 apparently benign epithelioma and 1 primary sarcoma. Reports of only 8 other cases of spontaneous gastric cancer in mice have been found in the literature (Stewart and Andervont<sup>23</sup>, Wells, Slye and Holmes<sup>22</sup>). Of many thousands of mice of different strains and ages many of them subjected to various experimental procedures employed in this laboratory, which have been examined post mortem during the past several years, not a mouse has shown a spontaneous or an induced malignant tumor of the stomach. Stewart and Andervont described an unusual type of adenomatous hyperplasia of the gastric mucous membrane occurring regularly in mice of strain I, but this lesion involves the glandular mucous membrane and is entirely different structurally from the induced tumors of the squamous mucosa of the forestomach dealt with in the present report.

#### SUMMARY

Squamous papilloma of the stomach was produced in 4 mice and squamous cell carcinoma of the stomach in 4 mice of strain A by injection of a solution of methylcholanthrene in liquid petrolatum into the anterior wall of the stomach of either or of both gastric chambers.

21 Passey, R. D. *J. Path. & Bact.* **47**: 349, 1938.

22 Wells, H. G., Slye, M., and Holmes, H. F. *Am. J. Cancer* **33**: 223, 1938.

23 Stewart, H. L., and Andervont, H. B. *Arch. Path.* **26**: 1009, 1938.

# STUDIES ON RESISTANCE TO TRANSMISSIBLE LEUKEMIA IN MICE BY MEANS OF PARABIOSIS

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Parabiosis offers an excellent means of investigating the role of humoral factors in resistance and susceptibility to transmissible neoplasms. Several investigators have already used this method in a search for circulating antibodies in the serums of laboratory animals resistant to transmissible neoplasms, but their results were contradictory. Transmissible leukemia of mice, a neoplasm of the blood-forming organs, is fatal after a definite duration of illness, characteristic for each strain. This permits a relatively accurate determination of factors that may modify the course of leukemia. We have undertaken a study of the hypothetical factors of resistance and susceptibility to leukemia by the method of parabiosis.

In 1863 Paul Bert, an anatomist, studying the problem of transplantation, united two rats by suturing together their skin, muscle and peritoneum (quoted by Møller-Christensen<sup>1</sup>). In 1908 Sauerbruch and Heyde<sup>2</sup> described an operation designed to make a permanent union between two animals by celioanastomosis, and named the condition resulting from this operation parabiosis. Rats were found to be most suitable, but guinea pigs, mice, rabbits, monkeys and other animals were also successfully united. Bunster and Meyer<sup>3</sup> modified Sauerbruch's technic by suturing the four leaves of the peritoneum together, thus eliminating a common peritoneal cavity and excluding herniation of intestinal contents from one animal to the other.

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1 Møller-Christensen, E. *Acta path et microbiol Scandinav*, 1935, supp 22, p 1.

2 Sauerbruch, F, and Heyde, M. *Munchen med Wchnschr* **55** 153, 1908.

3 Bunster, M, and Meyer, R. K. *Anat Rec* **57** 339, 1933.

Parabiosis has been used in studies of metabolism, immunity to infection, resistance to tumors and, more recently, hormones (Møller-Christensen<sup>1</sup>)

Several investigators have studied the problem of immunity to tumors by means of parabiosis, but the results are not uniform. Rous<sup>4</sup> united tumor-bearing rats with rats that proved resistant to three successive injections of the same tumor and noted no effect on the tumor growth. The results of Rous were confirmed by Morpurgo,<sup>5</sup> who transplanted sarcoma to susceptible rats that were connected with resistant ones. The resistant rats did not exert any influence on the tumor growth. Albrecht and Hecht<sup>6</sup> transplanted tumor into one of parabionts and found that the tumor grew more slowly in the parabiont than in single mice. They concluded from their experiments that the normal parabiont inhibited growth of the tumor transplanted in the parabiotic host. If they separated the mice, the tumor grew more rapidly. Lambert<sup>7</sup> united rats that were inoculated with mouse tumor with normal mice and found that the growth of the mouse tumor in the rats was promoted by this union. He assumed that this was brought about by passage of nutritive material from the mouse to the rat. Recently Fischer<sup>8</sup> united mice bearing spontaneous tumors with normal mice and observed acceleration of the tumor growth.

#### MATERIAL AND METHODS

The mice used in this study were members of stocks A, R and S, which have been inbred in this laboratory since 1928.<sup>9</sup> Mice of stock C were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The susceptibility of stocks A and R to different strains of leukemia has been established through numerous experiments. Mice of stock R are resistant to leukemia that originates in stock A but susceptible to leukemia appearing in stock R, and vice versa. Stock S is partially susceptible to leukemia occurring in stock A, and vice versa.

The interval between inoculation of leukemic cells and death from leukemia varies with different strains of leukemia. Most of the transmissible strains of lymphoid leukemia used in this study caused death of single susceptible animals approximately one to three weeks after inoculation. The myeloid and monocytic strains had a more chronic course. Parabiotic and control mice received, in the

4 Rous, P. *J. Exper. Med.* **11** 810, 1909.

5 Morpurgo, B. *Frankfurt Ztschr. f. Path.* **34** 341, 1926.

6 Albrecht, H., and Hecht, V. *Centralbl. f. allg. Path. u. path. Anat.* **20** 1039, 1909.

7 Lambert, E. A. *J. Exper. Med.* **13** 259, 1911.

8 Fischer, R. *Deutsche Ztschr. f. Chr.* **249** 616, 1938.

9 (a) Furth, J., Seibold, H. R., and Rathbone, R. R. *Am. J. Cancer* **19** 521, 1933. (b) Richter, M. N., and MacDowell, E. C. *J. Exper. Med.* **57** 1, 1932.

tail vein, 0.1 cc of a suspension of leukemic cells in Tyrode's solution, obtained usually from the spleen of a leukemic mouse. This suspension contained approximately 10,000 cells per cubic millimeter. Mice that died of diseases other than leukemia before the expected time of death from leukemia were omitted from the tables. Usually mice of the same sex, approximately 6 to 12 weeks of age, were united. In many instances, when one mouse died its parabiont was separated surgically and subsequently observed for a period of from several days to two months.

The technic used was that of Sauerbruch<sup>2</sup> as later modified by Bunster and Meyer<sup>3</sup>. Briefly, it consists in union of two animals by means of peritoneum, abdominal muscles and skin, with scapulas sutured together for firmer support.

In preliminary experiments it became apparent that shortly after operation the mice began to strain and tear the cephalad end of the suture line. In order to prevent this, a neckband of aluminum, measuring approximately 8 by 0.3 by 0.1 cm, partly covered with adhesive tape, was fitted around the neck of each mouse and joined at the time of operation.

#### EXPERIMENTS

The experiments will be described in four groups. In group 1 two mice susceptible to leukemia were united. In group 2 a susceptible and a resistant mouse were joined, and the susceptible mouse was inoculated with leukemic cells. In group 3, also consisting of paired susceptible and resistant animals, the resistant animal was inoculated. In group 4, susceptible mice were united with mice of a stock that was partially resistant to the strain of leukemia used, in some instances the partially resistant, in others the susceptible mouse, was inoculated.

*Inoculation of Parabiotic Twins Both of Which Were Susceptible to Leukemia*—Since a tumor graft is influenced by the general health of the animal, it seemed desirable to determine what effect, if any, union with another susceptible mouse would have on the course of leukemia in an inoculated susceptible animal as compared with an inoculated single mouse. This series of experiments would show also whether leukemic cells pass from the inoculated susceptible to the uninoculated susceptible mouse.

The results of the injection of leukemic cells from four different strains into one of two susceptible parabionts are shown in table 1. In 8 of the 9 parabiotic twins, both mice died of leukemia. The leukemic infiltrations were almost as advanced in the uninoculated as in the inoculated animal. The average length of life after inoculation of the parabiotic mice was approximately the same as that of single control mice given similar injections.

*Inoculation of Parabiotic Twins of Which One Mouse Was Susceptible, the Other Resistant*—(a) *Inoculation of the Susceptible Mouse*. These experiments were performed to determine whether the inhibiting factors present in the resistant animal would pass from the resistant uninoculated animal to the susceptible inoculated one,



thereby either preventing or delaying the development of leukemia. The susceptible mice were inoculated intravenously from one to twenty-seven days before and from three to seven days after parabiosis.

TABLE 1—*Inoculation of Susceptible Mouse in Parabiosis with Susceptible Mouse\**

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected†	Days Between Operation for Parabiosis and Injection	Result of Inoculation		Controls			
				Inoculated Mouse	Uninoculated Mouse	Number Inoculated	Number Showing Positive Result	Days of Life After Inoculation	
								Extremes	Average
26	C R♂-C R♂	Rfb 85	25	- D 42	D 42	10	9	D 31-4	29
31	AK R♀-AK R♀	Rfb 85	25	- D 28	- D 28	1	2	D 22-8	27.5
33	AK R♀-AK R♀	Rfb 85	21	+ D 18	- D 18	4	2	D 22-13	27.1
34	AK R♂-AK R♂	Ha 20	31	+ D 15	+ D 15	9	9	D 15-23	17
37	AK R♂-AK R♂	Ha 20	22	+ K 10	- K 10	9	9	D 15-23	17
39	AK R♀-AK R♀	Ha 20	18	+ D 17	+ D 17	9	9	D 15-23	17
42	AK R♀-AK R♀	Hb 4	20	+ D 26	+ D 26	4		D 24-26	25
45	AK R♀-AK R♀	Hb 4	14	+ D 24	- D 24	4		D 24-26	25
218	S♂-S♂	Sib 51	3	- K 11	- D 96	2	2	D 15-16	15.5

\* In all the tables the abbreviations used are as follows: C-R, hybrid mice between C and R mice, AK-R hybrid mice between AK and R mice, AK-R, AK mouse in parabiosis with R mouse. +, inoculation successful, -, inoculation unsuccessful. D, died; K, killed. The figures following D and K indicate the number of days after inoculation.

† Strain Rfb 85 is monocytic, Ha 20 and Hb 4 are lymphoid, and Sib 51 is myeloid (chloroleukemia).

§ This parabiont inoculated with chloroleukemia Sib 51 failed to show the disease although both control mice succumbed to it. This strain of chloroleukemia yields variable results and in occasional experiments inoculated mice remain healthy.

TABLE 2—*Inoculation of Susceptible Mouse in Parabiosis with Resistant Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Injection and Operation for Parabiosis	Result of Inoculation	
				Inoculated Susceptible Mouse, Days After Inoculation	Uninoculated Resistant Mouse, Days After Operation
109	AK R♀-R♀	AKf 5*	1	- D 13	- K 13
103	AK R♀-R♀	AKf 5	2	+ D 13	- D 13
104	AK R♀-R♀	AKf 5	2	+ D 11	- K 48
155	AK R♂-R♂	AKh 106†	24	+ K 39	- K 64
157	AK R♂-R♂	AKh 106	25	+ K 52	- K 39
181	AK R♀-R♀	AKh 106	25	+ D 69	- K 67
182	AK R♀-R♀	AKh 106	27	+ K 41	- K 41
Days Between Operation and Injection					
133	AKf♂-AK R♂	AKf 5	3	+ D 9	- K 70
130	AKf♂-AK R♂	AKf 5	7	+ D 7	- K 70

\* Strain AKf 5 is acute lymphoid leukemia.

† Strain AKh 106 is chronic myeloid leukemia.

was effected. It was believed that if the susceptible mice were inoculated before union the leukemic cells would multiply and would be more numerous at the time of operation, so that a large number could pass to the resistant parabiont.

Of the 7 susceptible parabionts inoculated from one to twenty-seven days before operation, 6 died with advanced leukemia from eleven to sixty-nine days after inoculation. Both susceptible mice inoculated three and seven days after operation died with leukemia nine and seven days, respectively, after inoculation. In no uninoculated resistant mouse did leukemia develop.

In each experiment with lymphoid leukemia, from 3 to 6 normal susceptible mice were inoculated. All died with leukemia from ten to thirteen days after inoculation.

Of 7 mice inoculated with myeloid cells of strain Akh 106 (controls to twins 155 and 157), 6 were killed at an advanced stage of the disease, forty-three days after inoculation. In a later experiment all 8 mice inoculated (controls to twins 181 and 182) were killed and showed advanced leukemia twenty-seven days after inoculation.

TABLE 3—*Inoculation of Resistant Mouse in Parabiosis with Susceptible Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Operation for Parabiosis and Injection	Result of Inoculation	
				Inoculated Resistant Mouse, Days After Inoculation	Uninoculated Susceptible Mouse, Days After Operation
93	Af♀-Ak♀	Akf 5	5	— K 21	+ D 21
96	Af♀-Ak♀	Akf 5	3	— D 27	— K 27
127	Af♀-Ak R♀	Akf 5	9	— K 15	— D 15
132	Af♂-Ak R♂	Akf 5	5	— K 70	— K 70
135	Af♀-Ak R♂	Akf 5	3	— K 70	+ D 28
129	Af♂-Ak R♂	Akf 5	7	— K 46	— K 46
136	Af♀-Ak R♀	Akf 5	2	— K 11	+ D 11
99	Ak R♀-R♀	Akf 5	1	— D 20	— D 20
178	Ak R♀-R♀	Akf 5	3	— K 19	+ K 19
179	Ak R♀-R♀	Akf 5	3	— D 13	+ D 41
184	Ak R♀-R♀	Akf 5	9	— K 36	— D 36

(b) Inoculation of the Resistant Mouse. The results summarized in table 3 show that in none of the resistant inoculated mice did leukemia subsequently develop whereas approximately one half of the uninoculated susceptible mice died of this disease from eleven to forty-one days after inoculation. Single susceptible mice inoculated with this strain died of leukemia from nine to eighteen days after inoculation.

The resistant inoculated mouse in pair 179 died thirteen days after inoculation. The susceptible parabiont was separated surgically and died of leukemia twenty-eight days after separation. The passage of malignant cells must have occurred within thirteen days after operation.

Previous experiments have shown that the length of life after inoculation is in inverse relation to the inoculating dose<sup>9b</sup>. The long and variable interval between inoculation of the resistant and death of the

uninoculated susceptible parabionts can be explained by assuming that most of the cells perished in the inoculated resistant mice and that only small variable numbers of leukemic cells entered the circulation of the susceptible mice

TABLE 4—*Inoculation of Mouse of Partially Resistant Stock in Parabiosis with Susceptible Mouse*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Operation for Parabiosis and Injection	Result of Inoculation	
				Inoculated Mouse of Partially Resistant Stock	Uninoculated Mouse of Susceptible Stock
116	Ak R♀-S♀	Akf 5	2	— D 14	— D 14
117	Ak R♀-S♀	Akf 5	2	— D 13	— K 13
118	Ak R♀-S♀	Akf 5	1	— D 12	— D 12
111	Ak R♀-S♀	Akf 5	6	— D 6	— D 36
141	Ak R♀-S♀	Akf 5	9	— K 53	— D 18
146	Ak R♀-S♀	Akf 5	2	+ D 18	+ D 18
147	Ak R♀-S♀	Akf 5	2	+ D 17	D 13
150	Ak R♀-S♀	Akf 5	10	— D 22	— D 22
164	Ak R♀-S♀	Akf 5	12	— D 51	— D 51

TABLE 5—*Inoculation of Susceptible Mouse in Parabiosis with Mouse of Partially Resistant Stock*

Pair	Stocks and Sex of Mice	Strain of Leukemia Injected	Days Between Injection and Operation	Result of Inoculation	
				Inoculated Mouse of Susceptible Stock	Uninoculated Mouse of Partially Resistant Stock
108	Ak R♀-S♀	Akf 5	Same day	+ D 13	— K 13
110	Ak R♀-S♀	Akf 5	2	+ D 11	— K 48
121	Ak R♀-S♀	Akf 5	3	+ D 10	— D 10
115	Ak R♀-S♀	Akf 5	7	+ D 9	— K 9
159	Ak R♀-S♀	Akh 106	22	+ D 38	— K 72
170	Ak R♀-S♀	Akh 106	23	+ D 62	— K 58
167	Ak R♀-S♀	Akh 106	34	+ D 77	— K 46
171	Ak R♀-S♀	Akh 106	23	+ D 61	+ D 31
Days Between Operation and Injection					
134	Ak R♀-S♀	Akf 5	3	+ D 9	— K 70
137	Ak R♀-S♀	Akf 5	2	+ D 9	— K 9
138	Ak R♀-S♀	Akf 5	8	+ D 9	— K 9
142	Ak R♀-S♀	Akf 5	6	+ D 13	— D 13

*Inoculation of Parabiotic Twins of Which One Mouse Was from a Susceptible, the Other from a Partially Resistant Stock*—At the time when the Ak R hybrids were paired with S mice we considered the S mice not susceptible to leukemia of stock Ak, but unexpectedly several of the inoculated S mice died of leukemia

(a) *Inoculation of Mouse of Partially Resistant Stock* The experiment included nine pairs of mice. Table 4 shows that the sus-

ceptible uninoculated parabionts of six pairs died of leukemia from thirteen to fifty-one days after inoculation of the partially resistant parabiont. Three of the inoculated, supposedly resistant parabionts also died of leukemia. In the remaining three pairs, 5 mice died and 1 was killed from twelve to fourteen days after inoculation and showed no evidence of leukemia.

The results of this experiment necessitated reinvestigation of the susceptibility of the S stock to lymphoid leukemia of stock Ak. Twenty-eight single mice of the S stock were inoculated, and 10 of these died of leukemia from nine to twenty-three days after inoculation.

The results of the experiments shown in table 4 indicate that of the S mice inoculated, some were susceptible and others were resistant to leukemia.

(b) Inoculation of the Susceptible Mouse. This group comprises twelve pairs of mice, in which 8 susceptible mice were inoculated before and 4 were inoculated after operation. Table 5 shows that all of the inoculated susceptible (Ak R) mice died with leukemia but that with a single exception the uninoculated S mice remained healthy.

#### OBSERVATIONS ON THE CIRCULATION BETWEEN PARABIOTIC MICE

Numerous investigators have attempted to demonstrate that there is a communication of lymph and blood capillaries between parabionts (Møller-Christensen<sup>1</sup>, Rossle<sup>10</sup>, Mayeda<sup>11</sup>, Sauerbruch and Heyde<sup>2</sup>, Morpurgo<sup>12</sup>). Sauerbruch and Heyde<sup>2</sup> injected iodine into a parabiotic rat and recovered it in the urine of the nontreated parabiont within forty-five minutes after the injection. Volumetric studies following intravenous or intracardiac injections of brilliant red (Hill<sup>13</sup>) showed that the dye is present in the same concentration in each of the parabiotic rats in approximately six hours. Immune bodies (Friedberger and Nasetti<sup>14</sup>) and bacteria (Sauerbruch and Heyde<sup>2</sup>, Ranzi and Ehrlich<sup>15</sup>, Steiner and Steinfeld<sup>16</sup>) pass from the parabiont into which dye was injected into the other parabiont. When India ink was injected into the heart of a parabiont (Goldman, quoted by Sauerbruch and Heyde<sup>2</sup>), both animals had deposits of black particles in liver and spleen.

10 Rossle, R. *Virchows Arch f path Anat* **300** 31, 1937

11 Mayeda, T. *Deutsche Ztschr f Chir* **167** 295, 1921

12 Morpurgo, B. *Verhandl d deutsch path Gesellsch* **14** 259, 1910

13 Hill, R. T. *J Exper Zool* **63** 203, 1932

14 Friedberger, E., and Nasetti. *Ztschr f Immunitätsforsch u exper Therap* **2** 509, 1909

15 Ranzi, E., and Ehrlich, H. *Ztschr f Immunitätsforsch u exper Therap* **3** 38, 1909

16 Steiner, G., and Steinfeld, J. *Klin Wchnschr* **6** 1597, 1927

Zacherl<sup>17</sup> showed that the leukocyte counts of the parabionts often differ conspicuously, and numerous observations of our own confirm this finding. The mechanism that maintains the numbers of leukocytes and erythrocytes in each parabiont is not overcome by the union, and it is not known to what extent vascular communications exist between parabiotic mice.

The passage of plasma between parabionts was investigated by injecting into one of the paired mice mouse or rabbit serum containing agglutinins against *Bacillus typhosus* and then titrating the agglutinins in the blood of both mice. The titers of the agglutinins in one pair four hours after the injection of the agglutinating serum are given in table 6.

The titers for a second pair were higher, but the ratios were similar.

Since immune globulins are among the largest substances in the serum, it can be assumed that hypothetic humoral substances inhibiting

TABLE 6—*Passage of Plasma Between Parabionts As Shown by Transfer of Agglutinins*

	Titer of Agglutination at Given Dilution of Serum					
	1:2	1:6	1:18	1:54	1:162	1:486
Parabiont given injection	+	++	—	—+	++	0
Parabiont not given injection	+	—	++	++	0	
Normal mouse (control)	0	0				

the growth of malignant tumors if present in the circulation of one mouse would pass freely to its parabiont.

The experiments described in which leukemic cells injected into resistant mice produced leukemia in their un inoculated susceptible parabionts indicate that white blood cells pass from one animal to the other.

Since the erythrocytes of a mouse are smaller than the leukocytes it seemed probable that they pass from one parabiont to the other in larger numbers than do the leukocytes. The passage of erythrocytes from one parabiont to the other was demonstrated in two ways.

1. Rat erythrocytes injected intravenously into one parabiont were demonstrated in the blood of the other by agglutination tests. A typical experiment was as follows:

Eleven days after establishment of parabiosis 0.3 cc of heparinized rat blood was injected intravenously into one parabiont, and thirty and sixty minutes later 0.5 cc was injected. Agglutination tests were made twenty minutes, one hundred and twenty minutes, twenty-four hours and forty-eight hours after the first injection with the serum of mice that had received repeated injections of washed rat erythrocytes. The results are given in table 7.

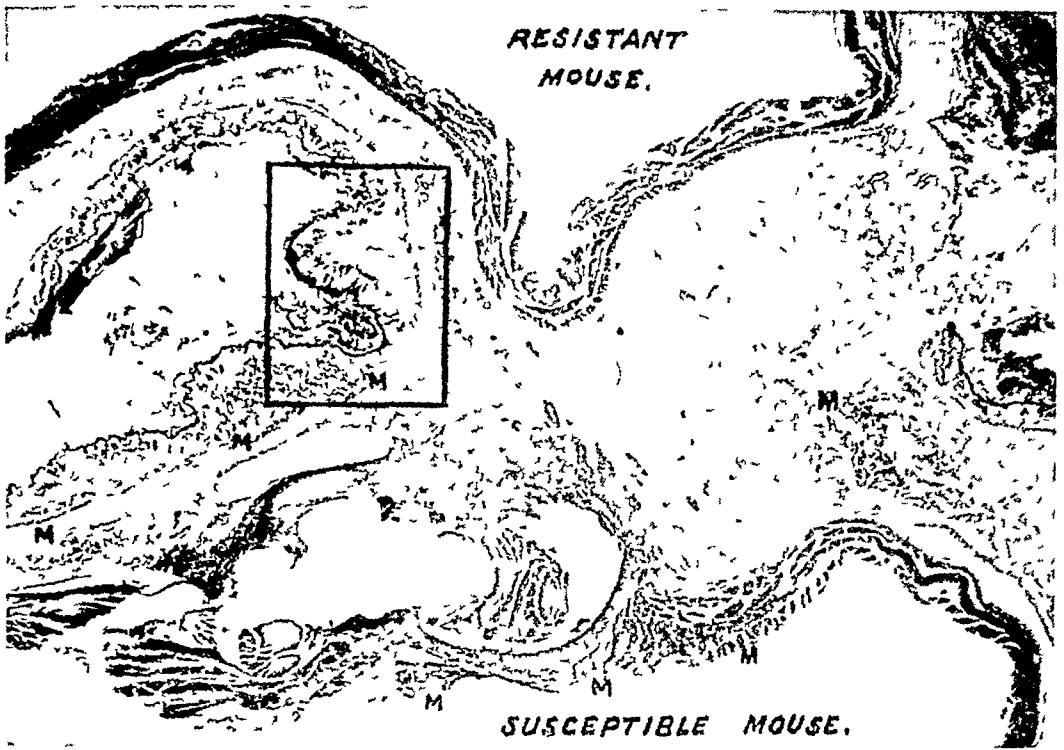


Fig 1—Site of anastomosis between a mouse with transmitted myeloid leukemia and a nonleukemic parabiont hematoxylin and eosin, approximately  $\times 15$  *M* indicates groups of immature myeloid cells in the tissues of the susceptible mouse. Such cells are absent in the tissues of the resistant mouse.

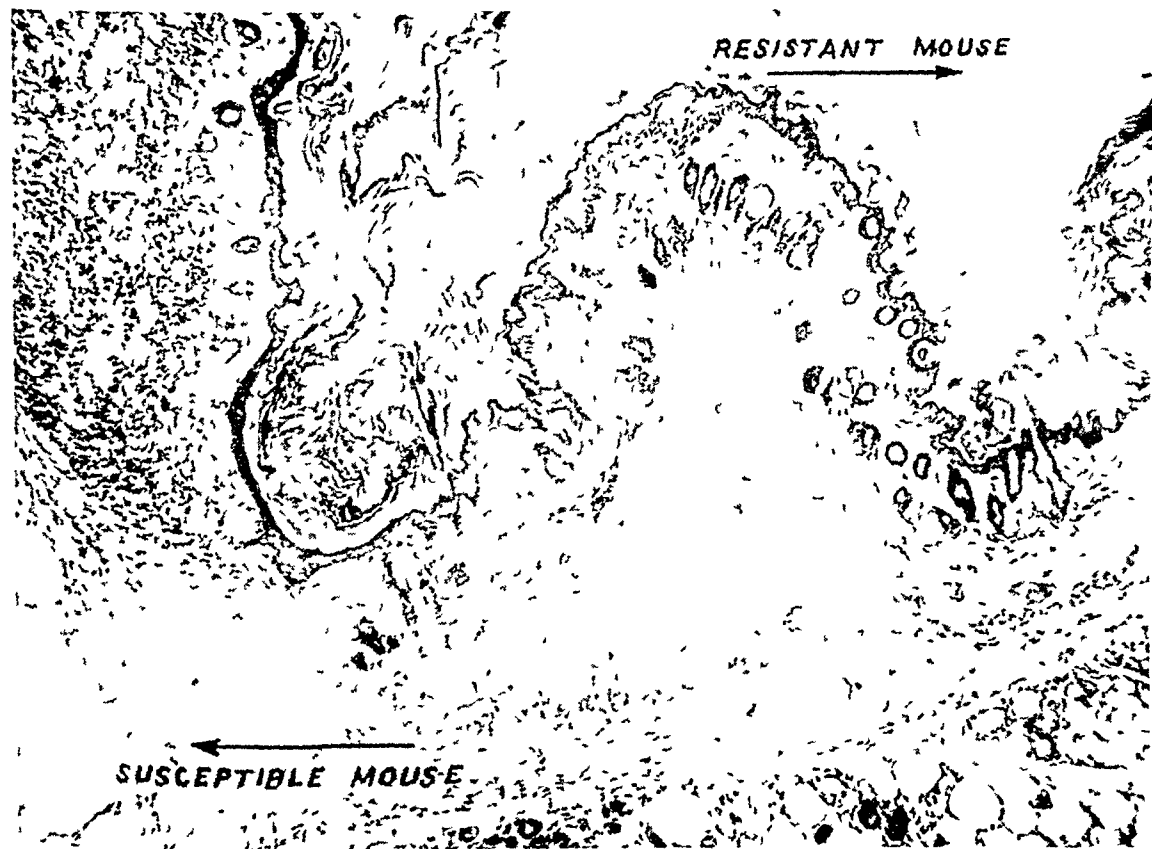


Fig 2—Higher magnification of the area indicated in figure 1, approximately  $\times 80$ . There is extensive infiltration by leukemic cells in the subcutaneous tissue of the susceptible mouse, but no leukemic cells are present in the neighboring tissue of the resistant mouse.

This experiment shows that rat erythrocytes were present in small numbers in the blood of the nontreated parabiont twenty minutes after its twin had received the first injection and large numbers of rat erythrocytes were present one hundred and twenty minutes after this first injection

2 When 0.3 cc of heparinized chicken blood was injected repeatedly intravenously into one member of a parabiotic pair eleven days after union, the typical nucleated oval avian erythrocytes were found in the organs of the mouse which had received no injection

Since erythrocytes are nonmotile, their presence in the mouse which had received no injection establishes the presence of blood vascular channels between parabiotic mice

Microscopic examination of the sites of anastomosis failed to show any extravascular migration of leukemic cells from one animal to the

TABLE 7—*Passage of Rat Erythrocytes Between Parabiotic Mice as Shown by Agglutination Tests*

Time After First Injection	Titer of Agglutination in			
	Parabionts		Controls	
	Mice Given Injection of Erythrocytes	Mice Not Given Injection	Rat Erythrocytes	Mouse Erythrocytes
20 minutes	+++	+	++ ++	0
120 minutes	+++	++	++ ++	0
24 hours	+++	+++	++ ++	0
48 hours	+++	+++	+++	0

other. Figures 1 and 2 show the site of anastomosis between a mouse with myeloid leukemia and a resistant mouse. There is perfect union of the skin. There is advanced leukemic infiltration in the subcutaneous tissue of the leukemic mouse close to the site of union, but the subcutaneous tissue of the resistant animal at a distance of a few hundred microns is free from infiltration. There are many capillaries and dilated lymphatics, as described by Rossle.<sup>10</sup>

#### COMMENT

When viable leukemic cells were inoculated into a susceptible mouse in parabiosis with another susceptible mouse, leukemia developed in both. Since it has been shown<sup>18</sup> that leukemia can be transmitted only by the introduction of viable leukemic cells, this observation indicates that the malignant cells passed from the inoculated to the uninoculated animal. The leukemic infiltrations of both the inoculated and the uninoculated mouse were about equally extensive, and both

18 Furth, J. J. *Exper. Med.* **61**: 423, 1935. Furth and others.<sup>9</sup>

died approximately the same number of days after inoculation as single susceptible control mice similarly inoculated. Hence parabiosis has no influence on the susceptibility to leukemia or on the length of life after inoculation.

When susceptible and resistant mice were paired and the former were inoculated with leukemic cells, leukemia developed in all but 1 of the susceptible and in none of the resistant mice. This shows that neither resistance nor susceptibility was conveyed from one parabiotic animal to the other. The life of the susceptible mouse was not prolonged by union with the resistant parabiont.

Inoculated resistant mice in parabiosis with susceptible mice failed to show leukemia, while in many of the susceptible uninoculated mice leukemia developed. This again indicates that neither susceptibility nor resistance was transferred from one parabiont to the other. In many instances viable malignant cells passed from the inoculated resistant to the uninoculated susceptible animal, as indicated by the development of leukemia in the latter. In other instances the leukemic cells were probably destroyed in the inoculated resistant mouse and viable leukemic cells failed to enter the susceptible animal.

Mice of a stock partially resistant to certain strains of leukemia did not acquire leukemia in a higher percentage of cases when inoculated with leukemic cells while joined in parabiosis with susceptible mice than when inoculated singly, nor was the course of the disease modified by this procedure. When partially resistant animals were joined to susceptible mice and the latter inoculated, leukemia developed in only a very small percentage of the partially resistant ones, although all the susceptible mice died of the disease. This indicates that the mice of the partially resistant stock in most cases were able to destroy the small numbers of leukemic cells that reached their circulation.

The present studies indicate that leukocytes pass from one animal to the other. The passage of erythrocytes is generally assumed, but the data in the literature do not indicate whether the transfer of substances from one parabiont to the other occurs by way of blood or by way of lymphatic channels. Microscopic sections show both capillaries and dilated lymphatics at the site of anastomosis. We have undertaken to demonstrate the passage of erythrocytes by blood vascular channels from one parabiont to the other. Rat erythrocytes are of approximately the same size as mouse erythrocytes, and their passage from one parabiont to the other can be demonstrated by agglutination with the serum of mice that have been immunized against rat erythrocytes. Subsequently we found that chicken erythrocytes can be demonstrated in the organs of one parabiont after injection of repeated doses into the other.



Since there is a communication of blood vessels between leukemic and resistant parabiotic mice small amounts of blood may constantly pass from the resistant to the leukemic animal and vice versa. Yet the duration of illness of mice inoculated with leukemic cells in parabiosis with mice resistant to leukemia was not prolonged as a result of parabiosis. Hence these experiments suggest that it is unlikely that the course of leukemia can be profoundly modified by continuous transfusion of normal blood.

These experiments demonstrate that resistance and susceptibility of mice to transmissible leukemia are not governed by humoral factors.

Our studies concern only inherited resistance to leukemia. It is possible that acquired resistance to neoplasms is associated with circulating antibodies.

#### SUMMARY

Parabiosis has been used as a means of studying the factors of resistance and susceptibility to transmissible leukemia in mice.

Leukemic cells pass from one susceptible parabiont to the other and produce leukemia in both.

The development of leukemia in a susceptible mouse inoculated with leukemic cells is not influenced by its parabiotic union with a resistant mouse.

Leukemic cells injected intravenously into a resistant mouse may pass to a susceptible parabiont producing leukemia in the latter without ill effect on the former.

Resistance to transmissible leukemia in mice of a stock only partially resistant to leukemia remains likewise unaltered by parabiosis with susceptible mice.

The presence of blood vascular anastomosis between parabionts has been demonstrated by the passage of erythrocytes from one parabiont to the other.

#### CONCLUSIONS

Inherited resistance and susceptibility to leukemia of mice are not transmitted from one parabiont to the other, hence it is unlikely that they are governed by humoral factors.

The passage of erythrocytes from one parabiont to the other indicates the presence of blood vascular anastomosis between parabiotic mice.

# REACTION OF MOUSE SKIN TO VARIOUS REDUCED AND PARTIALLY OXIDIZED SULFUR COMPOUNDS

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Mouse skin can be used to achieve that highly desirable result in studies of growth, the separation of various phases of growth and development from each other. Thus proliferation can be separated from cell differentiation, and this, in turn, from cell and tissue organization. Cells can be counted and measured, volumes of epiderm can be recorded, and so on. At least, this can be done anatomically, reasonable physiologic deductions also may be drawn, though at this and all other times it must be emphasized that, especially in incompletely differentiated cells, it is impossible to deduce physiologic states and possibilities from appearances. If the whole of growth and development is epigenetic and not preformed, so also is the development of just those kinds of cells which are most important in studies of both normal and abnormal growth, viz., incompletely differentiated cells. Their differentiations are developed, not merely unfolded, from manifold internal potencies determined and allowed to reach quantitative degrees by their environment.<sup>1</sup>

This separation of growth processes and these physiologic considerations are made seldom indeed, and this omission has led to utter confusion in meanings, a woeful lack of understanding among various workers and an impossibility of comparing results. We cannot emphasize too strongly that this lack of attention has caused and is causing a degree of confusion that is most regrettable.

We define cell differentiation as that series of propensities which cause a cell to rearrange its chemical makeup so that it becomes specific from nonspecific, loses its multiple competence or potency, gradually loses its power to multiply and takes its place anatomically and physiologically as part of a functioning tissue in an organism. Organization we define here as that series of processes by which cells of various differenti-

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<sup>1</sup> Weiss, P. Principles of Development, New York, Henry Holt & Co., 1939

ations gathered in integrated groups to form organs and parts which will and do function in an organism. Our views as to how malignant tendencies fit into this picture of growth and development have been expressed before.<sup>2</sup>

#### EXPERIMENTAL PROCEDURE

White petrolatum was used as the vehicle in which were incorporated 0.5 per cent thiocresol, cystine-disulfoxide, methionine sulfoxide, dl-methionine, cystine and cysteine sulfinic acid. Dibenzanthracene in 0.3 per cent mixture was also used as a sort of pathologic control. Each mixture was rubbed into the skin of the right ears and backs of 10 mice daily for three weeks. At the end of three weeks (eighteen applications) the ears and slices of skin were removed, fixed in Susa's solution, embedded in paraffin, cut at 7 microns and stained with hematoxylin and eosin. The images of the sections as projected from an Edinger apparatus were traced on paper, the lengths measured, and the area measured with a planimeter. The square millimeter area occupied under the aforementioned conditions by the skin was computed for 100 mm lengths. The standard deviations obtained from the raw data collected from the individual sections are used in computing the ratios. The compounds used were prepared and purified by Toennies and Lavine.<sup>3</sup>

	Thickness of Skin, Ratios
Controls (white petrolatum)	1
Thiocresol	3
Cystine disulfoxide	1.2
dl-methionine	1
Methionine sulfoxide	1
Cysteine sulfinic acid	1.1
Cystine	1
Dibenzanthracene	1.5

#### COMMENT

Thus far, no sulfur compound has been found which diminished the thickness of mouse skin, i. e., inhibited normal replacement proliferation. We draw no conclusions from this except that the experiments have not shown that the partially oxidized sulfur compounds used do not inhibit the rate of cell division of the skin, for we do not know at what rate desquamation occurred, and data on this are essential for evaluation of inhibition effects in this material. On the other hand, it has been shown that partially oxidized sulfur compounds do inhibit proliferation in special cases. Thus cystine disulfoxide inhibits the

<sup>2</sup> Reimann, S. P. *Biology of the Cancer Cell*, in *Symposium on Cancer Addresses Given at an Institute on Cancer Conducted by the Medical School of the University of Wisconsin, Madison, Wis.*, University of Wisconsin Press, 1938, p. 114.

<sup>3</sup> Toennies, G., and Lavine, T. F. *J. Biol. Chem.* **113**: 571, 1936. Lavine, T. F. *ibid.* **113**: 583, 1936. Toennies, G., and Kolb, J. J. *ibid.* **128**: 399, 1939.

proliferative phase in the growth of Obelia<sup>4</sup> It inhibits the rate of growth of spontaneous mammary tumors,<sup>5</sup> a result duplicated by English workers with partially oxidized sulfur groups in sulfanilamide types of compounds<sup>6</sup> Indeed, in connection with Hammett's<sup>7</sup> views on the physiologic activity of sulfur groups, it seems worthy of attention that the whole field of sulfanilamide therapy deals with suboxidized sulfur and with inhibition of proliferation<sup>8</sup>

Qualitatively there was no difference from the normal in the appearance of the skins rubbed with the partially oxidized compounds

In contrast, the sulfhydryl compound (thiocresol) had the usual effect of increasing the rate of cell proliferation, and thus there were produced more cells per unit of time per given area A description of the qualitative changes has been reported<sup>9</sup> and so will not be repeated, but several pertinent conclusions will be mentioned that were strengthened by these further experiments These are

- 1 The rate of cell multiplication can be speeded by application of a normal stimulus, viz, — SH

- 2 With this stimulation the cells proceed to higher degrees of differentiation and organization

- 3 Thus the potency or competence for differentiation and organization of cutaneous cells is greater than the normal realization of these processes

- 4 Multiplication in normal cells, in cells treated with sulfhydryl and cells treated with partially oxidized sulfur compounds occurs only in the basal layer

Fitted into the general knowledge of growth and development, the following remarks are pertinent There is direct and proportional antagonism between differentiation and multiplication<sup>1</sup> Tumors must arise from incompletely differentiated cells, therefore mostly from reserve cells or "spare parts" (The question of whether dedifferentiation of individual cells can occur will not be discussed here, though it is most pertinent<sup>1</sup>) An increase in the rate of cell multiplication alone does not lead to malignant growth<sup>9</sup> Cancer is not a disease of cell multiplication but one partly of cell differentiation and wholly of cell and tissue organization<sup>2</sup> Finally, we have been dissatisfied for some years with the classification which places benign and malignant

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4 Lavine, T F Am J Cancer **25** 809, 1935

5 Reimann, S P, and Hammett, F S Am J Cancer **26** 554, 1936

6 Boyland, E Biochem J **32** 1207, 1938

7 Hammett, F S Protoplasm **11** 382, 1930

8 Reimann, H A Arch Int Med **64** 362, 1939

9 Hammett, F S Protoplasm **13** 331, 1931 Reimann, S P Am J Cancer **15** 2149, 1931, Arch Path **17** 764, 1934

growths under one heading, viz, tumors, and the increasing number of "causes" being found for growths which have been classified for years as "benign tumors" crystallizes this sentiment when the causes are regarded in the light of the whole field of growth and development, including, as it does, embryonic and fetal growth and development, replacement, repair, physiologic hyperplasia and the other processes. We prefer calling benign tumors "growth anomalies," and we correlate them directly with such accepted anomalies of growth as the horseshoe kidney and the accessory spleen. Such anomalies are produced during embryonal times—but does growth stop when this period is concluded? Furthermore the old, purely anatomic factors cited in explanation of growth anomalies have been supplemented in many cases by physiologic ones in the form of organizers, gradients, fields and the like, to much benefit in experiment and correlation. Certainly the ordinary periductal fibroma of the breast has a physiologic cause so definitely known that its "cause" can be predicated on its mere presence in the breast under certain conditions<sup>10</sup>. And the mechanical causes are there, too just as they are assumed to have been present in the older less complete explanations of growth anomalies. At all events, benign growths have an organization, while malignant ones do not, and cellular differentiation in benign growths is quite different anatomically (and physiologically by presumption, for many reasons) from that in malignant growths. Therefore we give a definition of malignancy which was timidly advanced several years ago but which we now repeat with more assurance. "A malignancy is a mass of cells which arises from and continues to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative differences from the other cells of the organism with respect to the potencies of differentiation and organization particularly."

For an analysis of the dibenzanthracene-treated mouse skins from the present point of view see the previous report<sup>11</sup>.

Turning to the chemical aspects for leads and further explanations, we are confronted with many isolated facts. Probably the sulfur groupings do not stay as such when they are applied to cells, for the latter have broad capacities for bending the sulfur groupings into the locally required conditions, oxidizing or reducing them or establishing equilibria within wide limits according to internal and environmental conditions.

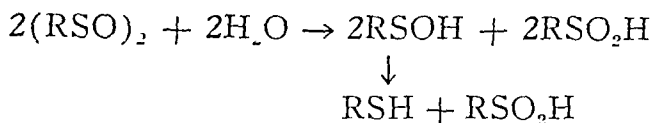
Cystine disulfoxide supported growth in weight in feeding experiments<sup>12</sup>. It also caused thickening of mouse skins a trifle through

10 Reimann, S. P. An Ovarian Tumor Diagnosed from a Breast Tumor in Libro de oro dedicado al Prof. Dr. Angel H. Roffo en ocasion de sus bodas de plata con la cancerologia, 1910-1935, Buenos Aires, 1935.

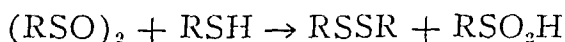
11 Reimann, S. P., and Chatalbash, N. Growth **1** 247, 1937.

12 Bennett, M. A. Biochem J **33** 885, 1939.

an increase in the number of cells. Presumably this is because it liberates sulfhydryl, for in the test tube cystine disulfoxide decomposes rather slowly into sulfhydryl and sulfinic acid



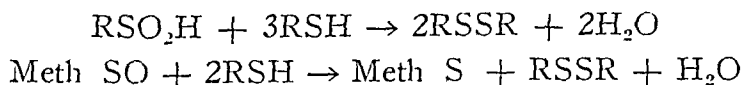
On the other hand, cystine disulfoxide inhibited the rate of growth of spontaneous mammary tumors in mice<sup>5</sup> and also the proliferative phase of the growth of *Obelia*.<sup>4</sup> Again, in the test tube cystine disulfoxide reacts instantaneously with sulfhydryl groups, thus applying brakes to proliferation



Of the two aforementioned possibilities, from the mass law it seems that in the case of dearth of sulfhydryl groups the first reaction would be favored, in the presence of sulfhydryl the second reaction would occur

Methionine may be converted to cystine and cysteine in the mammalian body.<sup>13</sup> It supports growth in weight and stimulates proliferation in *Obelia* but not in mouse skin. For this reason we conclude at present that the epiderm does not convert methionine to cysteine, for these experiments have shown that cystine does not increase skin proliferation, whereas cysteine does.<sup>14</sup>

Neither cysteine sulfinic acid nor methionine sulfoxide appears to inhibit tumor growth,<sup>15</sup> they cause no change in mouse skins, cysteine sulfinic acid does not support growth in weight, but methionine sulfoxide does.<sup>16</sup> Both compounds react very slowly with SH in the test tube.



Herein may be an explanation of the failure of proliferation-inhibiting effects

Neither cysteine sulfinic acid nor methionine sulfoxide yields sulfhydryl on decomposition. But the growth in weight evidence seems to indicate that methionine sulfoxide is readily shunted by the body into the normal pathways of methionine metabolism, whereas the body does not seem capable of utilizing the sulfinic acid similarly—this in spite

13 Toennies, G. *Growth* **1** 337, 1937

14 Brunsting, L. A., and Simonsen, D. G. *J. A. M. A.* **101** 1937, 1933

15 Preliminary experiments, to be pursued further, have demonstrated this

16 Bennett, M. A. *Biochem. J.*, to be published

of the fact that in vitro the reduction of the latter to cystine requires less energy than the reduction of methionine sulfoxide to methionine.

Unfortunately cystine disulfoxide, which is highly reactive with  $-SH$ , is also highly active in undergoing spontaneous decomposition, which leads to the inert compounds cystine and sulfinic acid<sup>3</sup>. The technical question arises, therefore, as to whether it is possible to discover or design a sulfoxide which will have high reactivity for sulfhydryl, and therefore proliferation-inhibiting properties, with, at the same time, a sufficiently increased resistance to spontaneous decomposition to permit it to have more than transitory existence in the body.

#### SUMMARY

Mouse skins have been rubbed with thiocresol, cysteine sulfinic acid, cystine, cystine disulfoxide, dl-methionine, methionine sulfoxide and dibenzanthracene. The sulfhydryl compound increased the rates of cell proliferation and the cells subsequently differentiated and organized to higher degrees than in normal skin. The other compounds showed no such effects. Some of the chemical relationships of these compounds are discussed in the light of their biologic behavior on mouse skin as well as in feeding and other experiments.

On correlating these findings with the general knowledge of growth and development, it is again concluded that malignancy begins in incompletely differentiated cells or "spare parts," that the changes of malignancy are qualitative changes in cell potencies and on the basis of these considerations and others a definition of a malignant growth is again proposed as follows. A malignant growth is a mass of cells which arise from and continue to proliferate within an organism as a result of and in direct proportion to their degree of internal qualitative difference from the other cells of the organism with respect particularly to the potencies of differentiation and organization.

# EFFECT OF DL-METHIONINE AND L-CYSTEINE ON THE CLEAVAGE RATE OF MAMMALIAN EGGS

BERNARD J MILLER, B S

AND

STANLEY P REIMANN, M D

PHILADELPHIA

Sulfhydryl has been shown to play a dominant role in cell division in about fifty species of animals and plants and also in many different processes in which cell division takes place. The experiments reported here were designed to show directly that sulfhydryl has the same effect of increasing rates of cell division in fertilized mammalian ova, the rabbit ovum being chosen as a test object. Other evidence that sulfhydryl performs this function in ova is that from the experiments of Pincus,<sup>1</sup> Gregory and Castle<sup>2</sup> and Gregory and Goss.<sup>3</sup> The last authors showed that there is a greater concentration of sulfhydryl groups as glutathione in the developing fetuses of giant strain rabbits and chickens than in the corresponding smaller size strains.

The use of sulfhydryl in these experiments requires exceptional chemical care, since this group is extremely labile, as one would expect it to be if it plays a dominant role in such a dynamic process as cell division. The technic and precautions have been outlined in extenso by Hammett,<sup>4</sup> particularly in connection with the analysis of the negative results obtained by some workers.

It is again emphasized that sulfhydryl affects only the proliferative phase of cell activity. What cells do further in the way of differentiation and organization are functions of other mechanisms (Hammett<sup>5</sup>). Obviously the early development of the fertilized rabbit ovum is mostly concerned with growth by proliferation. Differentiation in the ovum does not become evident until the middle of the blastocyst stage.

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From the Lankenau Hospital Research Institute

This investigation was aided by a grant from Mrs L. Elizabeth Nave

1 Pincus, G. The Metabolism of Ovarian Hormones Especially in Relation to the Growth of the Fertilized Ovum, in Cold Spring Harbor Symposia on Quantitative Biology, Cold Spring Harbor, L. I., New York, The Biological Laboratory, 1937, vol. 5, p. 44.

2 Gregory, P. W., and Castle, W. E. J. Exper. Zool. **59** 199, 1931.

3 Gregory, P. W., and Gross, H. J. Exper. Zool. **66** 155, 1933.

4 Hammett, F. S. Protoplasma **22** 489, 1934.

5 Hammett, F. S. Nature, London **141** 82, 1938.



It was first shown by Lewis and Gregory<sup>6</sup> and later by Pincus<sup>7</sup> that fertilized rabbit ova grown under ordinary conditions of tissue culture will cleave regularly up to the initial stages of blastocyst expansion and that they will collapse if cultured beyond this point. Ova grown in Carrel flasks or in the circulating cultures described by Pincus and Werthessen<sup>8</sup> will continue to expand beyond this point.

The compounds used in this study were dl-methionine, 99.7 per cent pure by analysis, prepared by C. S. Marvel at the University of Illinois and l-cysteine, 98 per cent pure by analysis, prepared by G. Toennies of this institute.

#### APPARATUS AND METHOD

Unicellular fertilized rabbit ova and ova in the morula of development were obtained by the usual flushing-out technic. Blood was first obtained from the doe by puncture of the heart and then stored overnight in the refrigerator. The serum was then obtained by centrifugation and stored in sterile tubes until the following day. In this manner it was possible to keep on hand sufficient fresh serum, approximately a day old. Serum older than two days was never used, although Pincus and Werthessen<sup>8</sup> claimed that serum as old as eleven days will still support the growth of the ovum. Bouillon cultures were always prepared for each batch of serum to insure against contamination.

Immediately after the cardiac puncture the animal was put to death, and the viscera were exposed under sterile conditions and the tubes and uterus excised. It is a wise precaution to insure against dust contamination from the air in the room by first spraying with 0.1 per cent phenol solution. The medium used for flushing consisted of equal parts of Tyrode's solution, which had previously been sterilized by filtration, and rabbit blood serum obtained from the doe killed the day before.

For ova younger than thirty hours after copulation flushing was usually done from the proximal portion of the tube with a sterile syringe. Ova in more advanced stages of development were best obtained by flushing from the fimbriated end of the tube. Washings were recovered in sterile watch crystals.

Within a few minutes the ova settle to the deepest portion of the watch crystal and may be easily located at a magnification of about 50. The ova are immediately transferred to the culturing apparatus by means of sterile pipets. Care must be exercised in this final step in order that damage to the ova may be avoided.

Two types of culturing devices were used. Watch crystals containing 1 or 2 cc of serum were placed in sterile moist chambers consisting of sterile Petri plate and a layer of moist filter paper placed in the bottom of the dish. Small and large Carrel flasks containing varying amounts of blood serum were also employed. These were used when growing blastocysts were to be observed.

Three series of experiments were conducted in order that accurate checks on each night be obtained. Unicellular ova were first cultured and the number of blastomeres recorded at definite intervals of time. By keeping the time constant one is able to observe the acceleration of cellular activity produced by sulfhydryl in relation to the rate of mitosis in control ova. Ova in the ten to twelve cell stage were next cultured and the morphologic characteristics of each ovum observed at

<sup>6</sup> Lewis W. H., and Gregory P. W. *Science* **69** 226, 1929.

<sup>7</sup> Pincus G. *The Eggs of Mammals* New York, The Macmillan Company 1936.

<sup>8</sup> Pincus, G. and Werthessen N. T. *J. Exper. Zool.* **78** 1, 1938.

the termination of the culture period. This method of observation was found to be more advantageous because it was less difficult to tell when an ovum was in the morula or blastula, than to count the number of cells beyond a certain point.

Other ova were studied in tissue culture by observing increase in diameter and morphologic changes over a longer period. Such ova were isolated in the eight to ten cell stage and cultured in small and large Carrel flasks. It was found that before the advent of blastocyst expansion the diameter of the enclosed mass of blastomeres increased more rapidly than the mean diameter of the ovum as measured from one surface of the zona pellucida to the other. It is obvious that the zona pellucida plays a scant part in growth and that it is the mass of blastomeres which is actually concerned with growth. It was therefore felt that measurements of the diameter of the cell mass rather than the diameter of the entire ovum would give a more accurate picture in relation to the growth of the fertilized ovum.

TABLE 1—*Stimulation of Cellular Activity in Ova Produced by dl-Methionine After Five Hours of Culturing*

Animal	Ova	Cells in Each	Medium	Cells at Conclusion of Experiment
R 8 A	6	1	Control of serum from animal R 7 A	2
		2		2*
		2		2*
		2	1 cc of serum containing 0.6 mg dl methionine	3*
		2		5
		2		5

\* This number indicates cellular activity

## RESULTS

In the first series of experiments six fertilized ova were recovered from doe R8A approximately twenty-six hours after copulation, five in the two cell stage and one as yet undivided. Three ova were placed in a watch crystal culture chamber containing 1 cc of blood serum from rabbit R7A as a control. The remaining ova were placed in another watch crystal chamber containing 1 cc of the same serum, to which had been added 0.6 mg of dl-methionine.

At the conclusion of a five hour period these ova were observed. The one control ovum which was unicellular at the beginning of the experiment had divided. The remaining two ova were still in the two cell stage, although both when studied closely showed evidences of dividing again.

Ova cultured in methionine were definitely stimulated. One ovum showed three distinct blastomeres and evidence of dividing again, while the remaining two ova contained five blastomeres each. The results of this experiment are shown in table 1.

Five unicellular fertilized ova were recovered from rabbit R9A approximately twenty-three hours after copulation. Two of these ova

were placed in a watch crystal culture chamber containing 1 cc of serum obtained from rabbit R8A as a control. The remaining three were placed in a watch crystal culture chamber containing 1 cc of the same blood serum, to which had been added 0.6 mg of dl-methionine. The results of twenty-two hours of culturing are given in table 2. Of the two ova placed in control culture, one continued to the four cell stage and the other to the two cell stage. Ova cultured in the presence of 0.6 mg dl-methionine in 1 cc of serum definitely showed acceleration of cellular activity and had already reached the six and eight cell stage with the exception of one which did not divide.

In the second series of experiments, ova in the eight to ten cell stage were recovered and cultured for twenty-two hours in watch crystal culture chambers. Observation has shown that ova older than thirty hours after copulation are less susceptible to "shock" than younger ova. At the

FIG 2—*Stimulation of Cellular Activity in Ova Produced by dl-Methionine After Twenty-Two Hours of Culturing*

Animal	Ova	Cells in Each	Medium	Cells at Conclusion of Experiment
R 9 A	5	{ 1 }	Control of serum from animal R 8 A	4
		{ 1 }		2
		{ 1 }	1 cc of serum containing 0.6 mg dl methionine	1
		{ 1 }		6
		{ 1*		8

\* A cleavage furrow was evident at the time of culture

conclusion of the experiment these ova were studied in order to determine what stage of development had been attained.

Seven ova in the eight to ten cell stage were recovered from doe R10A approximately fifty hours after copulation. Two ova were placed as a control in a watch crystal culture chamber which contained 1 cc of serum from doe NR9A. One ovum was placed in a hanging drop culture. Of the remaining ova two were placed in an identical culture chamber containing the same amount of blood serum, to which had been added 0.26 mg of l-cysteine and two in another culture chamber containing 0.52 mg of l-cysteine.

After twenty-two hours of culturing the two ova placed in a watch crystal culture were in the early morula of development (fig 1 A and E). The blastomeres were comparatively large and easily discernible, and as yet there were few signs of any peripheral flattening. The one ovum placed in a hanging drop was in the very early morula. The blastomeres were also large and few showed any signs of peripheral flattening.

The two ova cultured in the presence of 0.26 mg of l-cysteine were in the blastocyst when finally observed (fig 1 *A*). The blastomeres showed signs of differentiation. They were smaller than those of the control ova and far greater in number. The expanding vesicle and cleft

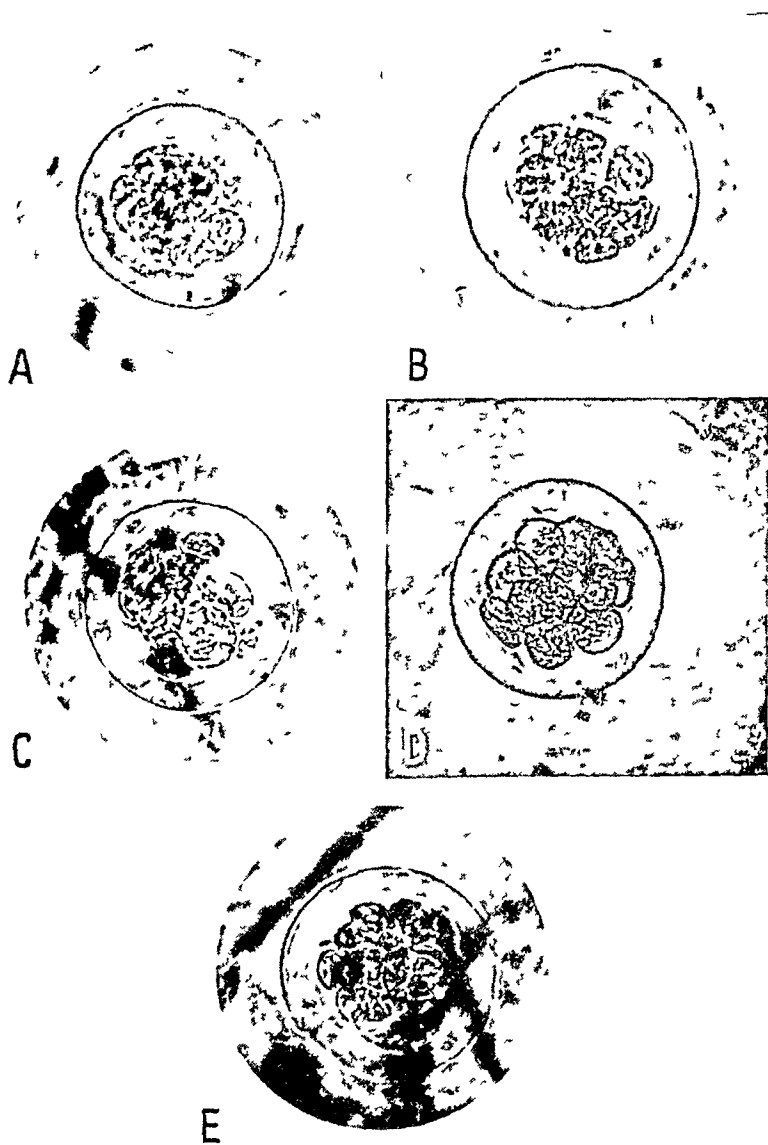


Fig 1—*A*, final appearance of ovum cultured in 1 cc of serum containing 0.26 mg of l-cysteine. *B*, final appearance of ovum cultured in 1 cc of serum containing 0.52 mg of l-cysteine. *C*, final appearance of second ovum cultured in 1 cc of serum containing 0.52 mg of l-cysteine. *D*, control ovum in hanging drop. *E*, control ovum in 1 cc of serum.

demarcating it were easily seen. As yet little expansion of the blastocyst cavity had occurred.

The remaining two ova, grown in culture containing 0.52 mg of l-cysteine, likewise showed stimulation of cellular activity (fig 1 *B* and

C) The blastomere of these ova were likewise smaller and more numerous than those of the control ova. However, these were not quite as far developed as those cultured in a medium containing 0.26 mg of l-cysteine.

The cultures were continued, and by the following day the control ova, excluding the one ovum in a hanging drop, had ceased developing beyond the very early morula. The ova cultured in cysteine had already collapsed, which indicated that these blastocysts had been expanding.

A third series of experiments were performed in which eight to ten cell ova were cultured in Carrel flasks for sixty-four hours. In this series increase in diameter of the enclosed cluster of blastomeres was noted and growth curves constructed from the data (fig 2). Two control ova from rabbit R10A were placed in small Carrel flasks containing 1 cc of rabbit serum. These ova never progressed beyond the morula. They did, however, show uniform growth up to this point. This is attributable to the fact that at this point the blastomeres were pressing against

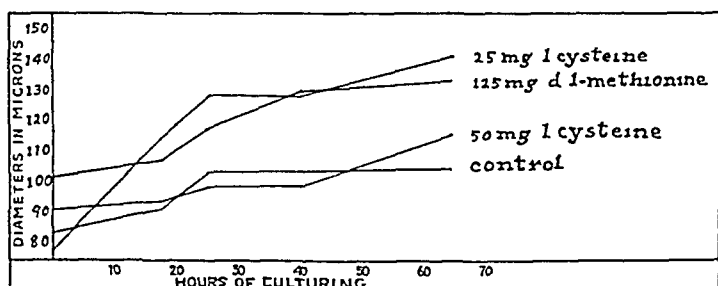


Fig 2—Curves of the proliferative growth of the blastomeres of rabbit ova when cultured in 1 cc of the serum of a doe to which various amounts of l-cysteine or dl-methionine had been added, as shown by the diameters of clusters of blastomeres at various intervals during the period of culture.

the internal surface of the zona pellucida, which really begins expanding only after the blastocyst begins to expand. As is seen in figure 2, the curve for the control ova is lowest.

Ova cultured in the same amount of blood serum containing 0.25 mg of cysteine showed rapid proliferation of blastomeres with consequent increase in diameter. This was most marked during the first twenty-five hours of culturing (fig 2). The average diameter of the enclosed cell mass remained unchanged during the following fifteen hours of culturing, after which another period of expansion began which lasted until the conclusion of the experiment.

When a concentration of 0.50 mg of cysteine was employed, there was little if any stimulation of blastomere proliferation. There was a slight increase in diameter during the first eighteen hours of culture. This was followed by a more rapid increase in diameter during the following seven hours. During the following fifteen hours the rate of increase

in diameter was considerably reduced. At the conclusion of the experiment these ova had obtained a peak far from that of ova cultured in a concentration of 0.25 mg of cysteine.

Ova cultured in the presence of 0.125 mg of dl-methionine showed a very definite increase in diameter during the entire time of culturing, terminating at a point lower than that observed with a concentration of 0.25 mg of l-cysteine.

#### COMMENT

Pincus<sup>1</sup> showed that glutathione, the tripeptide of cysteine, glycine and glutamic acid, added to circulating cultures of growing rabbit blastocysts, definitely causes an increase in blastocyst size. There is also a simultaneous decrease in the endometrial glutathione during the pre-implantation period of ovum growth, which is obviously attributable to the rapid utilization of glutathione by the growing ova and endometrium. Hammett,<sup>9</sup> in offering a possible explanation of the function of this naturally occurring molecule, has postulated that "nature has developed in one and the same chemical compound a complex which conditions if it does not determine the course of the several basic and essential processes concerned in developmental growth." The roles of the three amino acids of which glutathione is the derivation have already been adequately investigated by Hammett and reported in a long series of papers. According to this, then, the free sulfhydryl group of cysteine definitely stimulates the proliferative phase of growth, while glycine and glutamic acid are concerned with other phases of developmental growth which follow proliferation.

It seems possible, then, that in the growing ovum, as in other proliferating cells, sulfhydryl, the natural stimulant of growth, is made available to these dividing cells by utilization of naturally occurring glutathione. An analysis of figure 2 shows that a concentration of 0.25 mg of cysteine was most effective in producing an almost instantaneous increase in the rate of cellular activity in the proliferation phase of developmental growth of the fertilized rabbit ovum, while a concentration of 0.5 mg of cysteine was far less effective.

Methionine, like cysteine, definitely stimulates the proliferative phase of growth in *Obelia geniculata* (Hammett<sup>4</sup>), leaving the developmental phases of differentiation and organization unaffected. This same effect has been observed with growing rabbit ova (fig. 2).

These ideas are further substantiated by the fact that methionine and cysteine promote normal mammalian growth. Bennett<sup>10</sup> demonstrated that l-cystine could be replaced by methionine and various other sulfur-containing amino acids in the diet of rats.

9 Hammett, F. S. Science **79** 457, 1934.

10 Bennett, M. A. Biochem. J. **33** 885, 1939.

The possible chemical relationship between methionine and cystine in metabolism was first pointed out by Rose <sup>11</sup> when he showed that the role of cystine in growth is secondary to methionine

Hammett <sup>12</sup> showed that sulfhydryl is the essential stimulant to cell division. From this it seems evident that in the case of either an absence or a deficiency of sulfhydryl, methionine may serve as a source of free sulfhydryl groups. It is now definitely known that methionine can go to cystine or to cysteine and that this reaction is not reversible. This was directly proved when Tarver and Schmidt <sup>13</sup> demonstrated that radioactive sulfur ( $S^{35}$ ) when fed to rats as methionine may appear as cystine.

#### SUMMARY

Ova cultured in watch crystal cultures will collapse if cultured beyond the initial stages of the blastula. Expansion of the blastocyst can be observed when ova are cultured in Carrel flasks containing 2 to 3 cc of rabbit serum.

Unicellular rabbit ova, isolated before twenty hours after copulation, usually will not grow in tissue culture.

The diameter of the enclosed mass of blastomeres increases more rapidly in diameter than does the entire ovum as measured from one surface of the zona pellucida to the other. Ova cultured in 1 cc of blood serum containing 0.6 mg of dl-methionine show a more rapid increase in the number of blastomeres over a period of five hours than do ova grown as controls. Ova in two cell condition are likewise stimulated to rapid blastomere proliferation.

Ova cultured in 1 cc of blood serum containing 0.26 mg of l-cysteine show an immediate and rapid increase in blastomere proliferation, while ova cultured in the same amount of blood serum containing 0.52 mg of l-cysteine are not nearly so effectively stimulated. Ova cultured in Carrel flasks show a rapid increase in diameter when 0.25 mg of l-cysteine is added to each cubic centimeter of fresh rabbit blood serum. Ova cultured under the same conditions in the presence of 0.50 mg of l-cysteine are not so effectively stimulated to increase in diameter. Ova grown under the same conditions in the presence of 0.125 mg of dl-methionine were also stimulated and showed a definite increase in diameter.

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11 Rose, W. C., Kemmerer, K. S., Womack, M., Mertz, E. T., Gunther, J. K., McCoy, R. H., and Meyer, C. E. *J. Biol. Chem.* **114** 1445, 1936.

12 Hammett, F. S. *Protoplasm* **7** 297, 1929.

13 Tarver, H., and Schmidt, C. L. A. *J. Biol. Chem.* **130** 67, 1939.

# MORPHOLOGIC APPEARANCES OF SPIROCHETAL REPRODUCTION IN TISSUES

GABRIEL STEINER, M D

DETROIT

Certain morphologic appearances of spirochetes will be described that are interpreted as phenomena connected with the reproduction of these organisms in living tissues. In addition, evidence will be presented which indicates that there is a particular tissue reaction that depends on a characteristic phenomenon of spirochetal reproduction.

Spirochetal reproduction in the tissues can be studied best in (1) the tissues of patients in the very early stages of a spirochetal disease, during which time a large number of spirochetes are present—e g, the tissues of patients with congenital syphilis and the tissues of patients with untreated acute dementia paralytica—and (2) the organs of animal carriers chronically infected with spirochetes, e g, rats with leptospiral infection.

In several spirochetal diseases, the stage immediately before the crisis is characterized by the presence in the blood stream of densely clustered masses of spirochetes. These are called agglomerations. In mice and rats infected with the organism of relapsing fever these agglomerations appearing in the blood stream mark the end of a relapse (fig 1 A). Again, just prior to the termination of the disease similar agglomerations are seen in chickens infected with *Spirochaeta* (or *Borrelia*) *gallinarum*. In both of these diseases (relapsing fever and spirochetosis *gallinarum*) high serologic immunity develops, this immunity is probably related to the phenomenon of agglomeration.

As can be seen in dark field preparations and also in silver mirror preparations of blood smears<sup>1</sup> in the very early stages of experimental relapsing fever, the spirochetes divide by transverse fission. The spirochetes of relapsing fever and of spirochetosis *gallinarum* are prone to reproduce in the blood stream, and only in very late stages of these diseases do they invade parenchymal parts. When they are no longer able to live in the blood stream, they may still exist in the parenchyma of the central nervous system. The period of their persistence in the brain is very short in spirochetosis *gallinarum*. However, in experi-

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1 Steiner, G. J. Lab & Clin Med **23** 293, 1937



mental relapsing fever in mice, as has been shown by Buschke and K100,<sup>2</sup> Jahnel,<sup>3</sup> myself<sup>4</sup> and others, the spirochetes may persist in the brain as single individuals for a long period despite a high content of antibodies in the serum. During this time, which lasts for a year or more, the mice are immune to repeated inoculations. The same effect is seen in cases of dementia paralytica in which the treatment has included inoculation with the spirochetes of relapsing fever (*Borrelia duttoni*), borrelias are found in the brain for a rather long time after inoculation, although they are no longer present in the blood stream.<sup>5</sup> There seems to be no doubt that the brain affords some kind of protection against the effects of immune bodies of the serum during a time when the organisms cannot live or multiply in the blood stream. These residual spirochetes of relapsing fever as long as they remain in the brain have lost their power of reproduction. However, if emulsions of brain containing the spirochetes are inoculated into normal mice, reproduction will again take place. So, although the immunologic properties may be sufficient to prevent reproduction of spirochetes in the brain, these protective properties do not suffice to kill all of the micro-organisms.

It is not my intention to deal here with the factors responsible for the peculiar protection of spirochetes in the tissues of the central nervous system against the killing effects of immunologic substances in the serum.<sup>6</sup> In this paper interest is centered about the morphologic appearances in the tissues which result when the local reproduction of spirochetes is initiated.

My material consisted of (1) 3 cases of congenital syphilis in which tremendous numbers of spirochetes were seen in many organs of the body, (2) 58 cases of dementia paralytica, among which were 3 examples of early acute untreated stages of this disease, and (3) the kidneys and other organs of 10 rats naturally infected with *Leptospira icterohaemorrhagiae*. The organs were embedded in paraffin and stained for spirochetes by the gum mastic silver method.<sup>7</sup> Liquid cultures of spirochetes were made solid by use of gentle heat and then cut as solid blocks in frozen sections. When possible, smears and dark field examinations were made.

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2 Buschke, A, and Kroo, H. *Klin Wchnschr* **1** 2470, 1922

3 Jahnel, F. *Munchen med Wchnschr* **73** 2015, 1926

4 Steiner, G, and Schauder, H. *Klin Wchnschr* **4** 2288, 1925

5 Steiner, G. *Krankheitserreger und Gewebsbefund bei multipler Sklerose. Vergleichend-histologisch-parasitologische Untersuchungen bei multipler Sklerose und anderen Spirochätosen*, Berlin, Julius Springer, 1931, pp 64-65

6 Steiner, G, and Steinfeld, J. *Klin Wchnschr* **4** 1995, 1925, **5** 650, 1926

7 Steiner, G. *J Lab & Clin Med* **25** 204, 1939

In the cases of congenital syphilis the organs (liver, kidney, adrenal, lung and other organs) revealed immense numbers of spirochetes. Despite their numbers, the method of division of the individual spirochete could not be made out in the fixed tissues. It is certain, however, that the huge number of spirochetes seen in the organs must

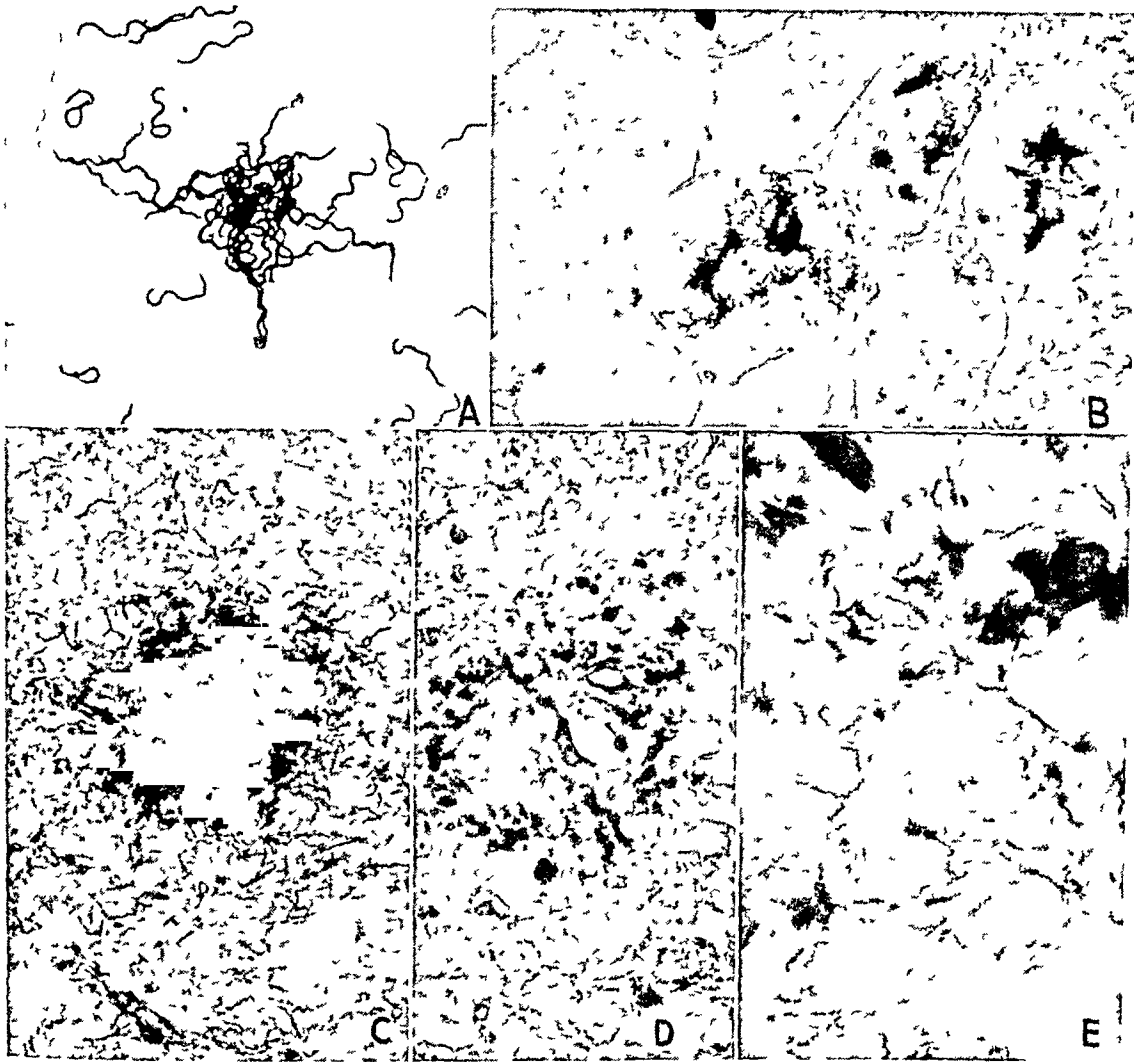


Fig 1—*A*, blood smear of a mouse with relapsing fever (*Borrelia duttoni*), showing an agglomeration of spirochetes at the end of a relapse. Silver mirror method,  $\times 1,000$ . *B*, kidney of a rat naturally infected with *Leptospira icterohaemorrhagiae*. The massive centers of leptospiras are seen limited to the tubules. Gum mastic silver method,  $\times 350$ . *C*, cerebral cortex in a case of acute dementia paralytica, showing a reproductive center (conglomeration), gum mastic silver method,  $\times 450$ . Note the spread of spirochetes from the center and the pericolumnal liquefaction of tissue. *D*, spirochetal reproductive center in the anterior lobe of the pituitary in a case of congenital syphilis, gum mastic silver method,  $\times 300$ . A yellow center is shown, and the peripheral zones consist of a black ring with degenerating spirochetes and granules of spirochetal debris. In the surrounding tissues numerous well preserved spirochetes are seen. *E*, higher magnification of a neighboring area, surrounding the reproductive center of *D*,  $\times 675$ .

have been the result of rather rapid spirochetal reproduction. In the tissues the organisms are distributed in two ways: (1) they are diffusely scattered, (2) they are accumulated in dense ball-like masses. It is to the latter appearance that I wish to draw special attention. Morphologically these ball-like masses are round or oval accumulations, made up of spirochetes closely packed together. No space for tissue elements seem to be left. With silver stains a characteristic feature of these large spirochetal masses is seen, the central and inner parts of the masses have a light yellow or brownish color, while the outer zone is black. The outer zone shows the usual black silver mirror appearance of spirochetes. Under the low power of the microscope these peripheral spirochetal coils tend to form a stellate pattern. At the periphery the spirochetes are arranged in raylike strands, the axes of which radiate from the center of the ball. The rays of this formation are made up of well defined black spirochetes. The central part of the mass consists of a compact yellow or brownish material which, by using a high power of the microscope, is seen to be formed by very fine, lightly stained spirochetal threads that are infinitely tangled. Such spirochetal conglomerations were seen in the liver, adrenals, hypophysis, intestinal walls and other organs in these cases of congenital syphilis.

As early as 1907 Benda<sup>8</sup> discovered massive conglomerations of spirochetes in the organs in cases of congenital syphilis, he called them "centers." Later on such conglomerations were seen by numerous pathologists. In 1906 Straussler<sup>9</sup> described peculiar multiple miliary areas of necrosis in the cerebral cortex in a case of dementia paralytica, and later Gruetter,<sup>10</sup> Hauptmann,<sup>11</sup> Heischmann<sup>12</sup> and Schob<sup>13</sup> demonstrated balls of spirochetes as the causes of these necroses. In my own 3 cases of early dementia paralytica conglomerations of spirochetes were also seen. In acute dementia paralytica the spirochetes were distributed in exactly the same way as has been described for congenital syphilis.

For the following reasons I believe that such conglomerations represent *centers of spirochetal reproduction*.

1. The conglomerations are seen only in recent stages of active syphilis, both in congenital syphilis and in early and acute stages of dementia paralytica. They are never found in chronic syphilis. In tertiary lesions, where spirochetes are scanty or absent, the conglomerations are never seen.

8 Benda, C. Verhandl. d. deutsch. path. Gesellsch. **23** 256, 1928.

9 Straussler, E. Monatschr. f. Psychiat. u. Neurol. **19** 244, 1906.

10 Gruetter, E. Ztschr. f. d. ges. Neurol. u. Psychiat. **54** 225, 1925.

11 Hauptmann, A. Monatschr. f. Psychiat. u. Neurol. **45** 59, 1919.

12 Heischmann, H. Ztschr. f. d. ges. Neurol. u. Psychiat. **55** 27, 1920.

13 Schob, F. Ztschr. f. d. ges. Neurol. u. Psychiat. **95** 588, 1925.

2 The finding of conglomerations is always combined with that of a diffuse distribution of very numerous spirochetes in the neighborhood of the massive centers. Thus a very active phase of reproduction is indicated. In congenital syphilis conglomerations are found in different organs in the same case. In dementia paralytica, if spirochetal conglomerations are seen, they are usually found in large numbers in the cortical or other gray areas, always together with innumerable diffusely distributed spirochetes. From these centers a diffuse penetration of spirochetes into the neighboring tissue takes place.

3 In colonies from cultures of spirochetes in artificial mediums it has been possible to demonstrate a striking resemblance to the appearance of the conglomeration centers in congenital syphilis and dementia paralytica. First, sterile calf serum was coagulated in a sterile tube. Then, with a needle, a puncture canal was made in the center of the coagulated cylinder of serum. Next, blood of a mouse inoculated with the spirochetes of relapsing fever was diluted to 1:100,000 with physiologic solution of sodium chloride, and 2 cc of this dilution was added to the tube with the coagulated serum. The tube was then centrifuged for five minutes and after that incubated at 30°C for three days. The contents of the tube were then fixed in 10 per cent solution of formaldehyde U.S.P. and cut like ordinary tissue blocks. In some of these sections, after staining with silver nitrate, one or two star-shaped colonies were apparent grossly. Microscopically, these colonies showed yellowish centers from which strands of shiny black spirochetes radiated peripherally (fig. 2). The central parts showed innumerable spirochetes having only a yellow color.

There can be no doubt that these spirochetal balls from the cultures represent colonies or centers of reproduction. They are exactly like the spirochetal balls or conglomerations seen in congenital syphilis and dementia paralytica. The conclusion seems justified that in these syphilitic diseases with very active spirochetal reproduction the conglomerations represent the same features of reproduction as do those in artificial solid mediums. Cultures in fluid mediums may show similar conglomerations, but usually the colonies are smaller and do not contain such large numbers of spirochetes. Apparently the physical resistance to the expansion of spirochetes during their reproduction is lower in fluid mediums than in solid or semisolid mediums. Therefore, numerous small conglomerations may be seen in fluid mediums, while in solid or semisolid mediums larger but less numerous colonies may be produced. Another feature of this type of reproduction of spirochetes in solid mediums has been observed: liquefaction of the medium in the immediate neighborhood of the spirochetal reproductive colony goes on. It may be of interest to note that the reproductive colonies

in tissues from syphilitic patients usually show the same phenomenon of circumcolonial liquefaction (Compare figure 2, an artificial culture, and figure 1 C, a conglomeration of spirochetes in cortex from a patient with dementia paralytica)

4 Similar conglomerations of leptospiras were seen in the kidneys of rats naturally infected with these organisms. These conglomerations were found only in the renal tubules. The glomeruli and other parts



Fig 2—Culture of the spirochetes of relapsing fever (*Borrelia duttoni*) in coagulated serum,  $\times 1,000$ . Note the conglomeration and the pericolonial liquefaction.

of renal structures did not show any leptospiras. In some places the masses were so large as to occlude the lumen completely, in other places the organisms were seen between the epithelial cells of the tubules (fig 1 B).

In silver preparations the inner parts of the large leptospiral conglomerations showed a light yellow color, while the outer parts were formed by black leptospira. So the same picture as seen in spirochetal

colonies in syphilis and relapsing fever appears also in leptospiral infections. One difference was noted, however. The colony has a hollow center which corresponds to the lumen of the renal tubule. Apparently the organisms are washed off by the flow of urine.

The conglomerative appearance of colonies in tissues and the appearance of similar colonies in solid artificial culture mediums seem to indicate that spirochetal reproduction under certain circumstances results in the formation of a characteristic morphologic picture.

The tissue reaction which results from the conglomeration of spirochetes is local miliaire necrosis. This type of necrosis is seen in the organs in congenital syphilis (liver, lung, pancreas, adrenal, vascular walls and other tissues) and in the gray matter of the brain in dementia paralytica.

To describe the lesions of congenital syphilis many confusing terms have been used. Some of these are miliaire necrosis, miliaire granuloma, miliaire abscess-like formation (without liquefaction), miliaire syphiloma and miliaire gumma. There are at least three different circumscribed histologic appearances in congenital syphilis: miliaire abscess-like formation, miliaire necrosis and miliaire granuloma. The so-called abscesses of Dubois in the thymus in congenital syphilis are necroses with secondary invasion of the necrotic masses by polymorphonuclear leukocytes. Miliare necroses with secondary invasion by leukocytes are also found in other organs in congenital syphilis. In all instances the necrosis is the primary lesion and not, as was formerly believed, necrosis of the central zone of a granulomatous reaction. The necrosis produced by the conglomeration of spirochetes is of a coagulation type. At the outset no formation of granulomatous tissue can be seen in these necrotic areas. Later they may develop into miliaire syphilomas, which then may show central necrosis surrounded by granulomatous tissue. But such a sequence has not definitely been established. At any rate, miliaire and submiliare necrosis with or without the secondary invasion of leukocytes is the histologic parallel of spirochetal conglomerations. This was first found by Benda<sup>8</sup> and since then by numerous other investigators. In the hypophysis pure necroses were seen, also.<sup>14</sup> In 1928 Schneider<sup>15</sup> stated that the relationship of this type of necrosis in the pituitary to the spirochetes had not been examined.

The anterior lobe of the pituitary in 1 of the 3 cases of congenital syphilis in my material showed spirochetal conglomerations in silver

14 Schmidt, M. B. *Verhandl. d. deutsch. path. Gesellsch.* 6: 207, 1903. Simmonds, M. *Dermat. Wchnschr. (suppl.)* 58: 104, 1914. Schmidt, P. *Centralbl. f. allg. Path. u. path. Anat.* 34: 466, 1923. Kraus, cited by Schneider,<sup>15</sup> p. 233.

15 Schneider, P. *Verhandl. d. deutsch. path. Gesellsch.* 23: 177, 1928.

preparations and miliary necroses in sections stained with hematoxylin and eosin. In order to establish the relationship of the spirochetal colonies to the miliary necrosis, paraffin sections stained by the gum mastic silver method<sup>7</sup> were made free of silver by diluted nitric acid, washed, and then stained with hematoxylin-eosin or cresyl violet. By this method the areas of miliary necrosis have been identified with the spirochetal conglomerations (fig 1 *D* and *E*). Serial sections, one

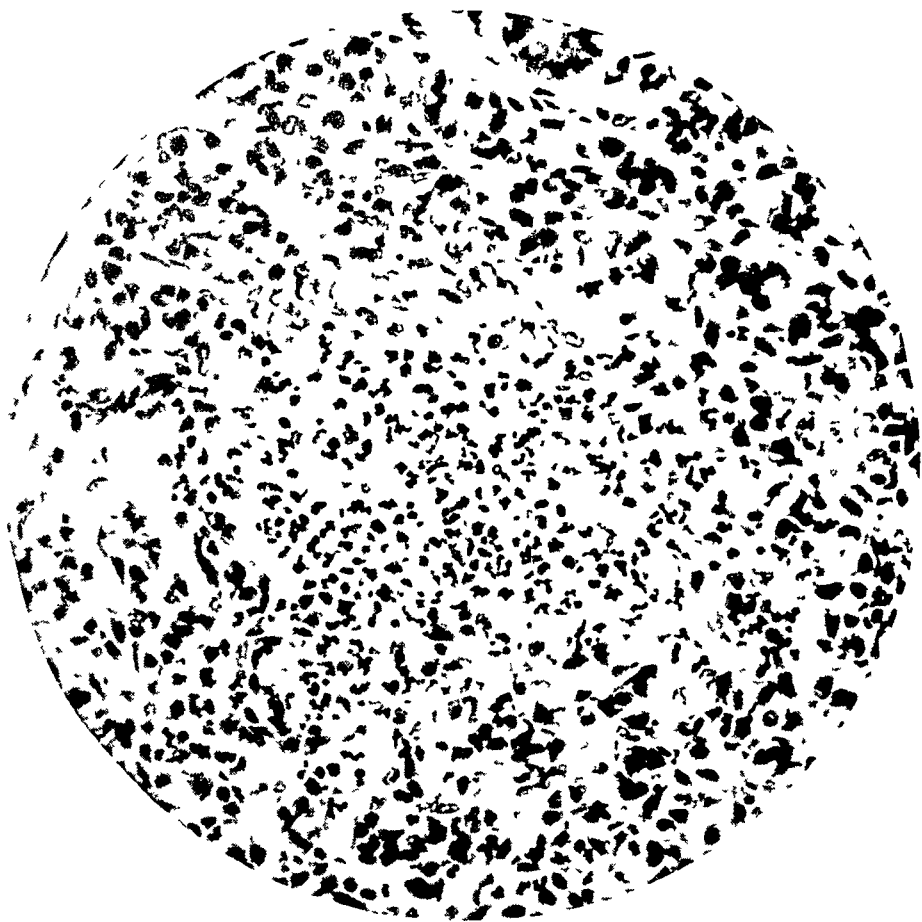


Fig 3—Submiliary necrosis in the anterior lobe of the pituitary, same section as figure 1 *D*, made free of silver and stained with cresyl violet,  $\times 300$ . Note invasion by polymorphonuclears.

stained with hematoxylin-eosin, the next stained by the gum mastic silver method, showed the same relationship between spirochetal conglomerations and miliary necroses. Occasionally an invasion of polymorphonuclears into the necroses or conglomerations was found (fig 3). This represents a transition toward abscess formation.

Primary necroses in organs in cases of congenital syphilis and in the brain in dementia paralytica are the immediate consequence of spiro-

chetal conglomerations, which themselves result from the very active reproduction of the causative agent in the tissues of the host

#### COMMENT

Miliary necroses in the tissues, as well as massive conglomerations of spirochetes (Benda's conglomerative centers), have been recognized as peculiar and rather characteristic lesions in congenital syphilis. Miliary necroses in the gray matter of the brain are also rather frequently demonstrated in dementia paralytica. In both instances the immediate relationship between these spirochetal conglomerations and the subsequent necroses has been established. That the miliary necroses or transitional stages between these miliary necroses and abscesses in the pituitary are due to spirochetal conglomerations is a new observation. The spirochetal conglomerations must be considered as reproductive centers, they are colonies of organisms in the living tissues of the host. The reasons for their occurrence are already given. The contention that the conglomerative phenomenon is not a postmortem effect is substantiated by the necrobiotic changes in the tissue at the same place. The histologic necrobioses are immediate intravital consequences of the spirochetal conglomerations. That the spirochetal conglomerations are not simply a part of a terminal stage of the infection may be demonstrated in 1 of the 3 cases of dementia paralytica. Here sudden death occurred, caused by epileptiform attacks, and the autopsy was done shortly after death. The spirochetal conglomerations in this case were the same as those found in the other 2 cases of dementia paralytica. Furthermore, there is no probability that the uniform pictures of spirochetal conglomerations found in many different organs in the same case of congenital syphilis are terminal effects. In dementia paralytica the difference in size and shape of spirochetal colonies indicates various evolutionary stages of spirochetal reproduction.

Reproductive colonies are found only in very acute stages of syphilitic diseases. The structure of these colonies in the tissues has an appearance which is almost identical with that of colonies growing in solid mediums. Furthermore, in the final stages of some spirochetal diseases characterized by the agglomerative phase of spirochetal reproduction (relapsing fever and spirochetosis gallinarum) numerous single *degenerating* spirochetes are almost always found. Degenerating spirochetes are recognized by the presence of spherical granules on one or both ends of the individual organism, by deformed spirals, by rings or loops, by parts fused together or even by isolated granules. Such degenerated forms are not seen in recent conglomerations or in their vicinity, where spirochetes are always rich in number.



In the conglomerative mass the center is the oldest part. The conglomeration grows by apposition of newly formed spirochetes, but the center does not show any signs of definite spirochetal degeneration. There is a difference in color, but this cannot be considered as a definite degenerative phenomenon. In this regard it would be of interest to follow the different histologic changes of the milium necroses and the parasitologic findings in them at different evolutionary periods of disease. In congenital syphilis older types of necroses, which are characterized by the invasion of leukocytes, occasionally are completely free of spirochetes, or the outer periphery may be marked by a circle of black granules and a few single spirochetes while the surrounding tissues contain very numerous diffusely distributed single spirochetes (fig 1 D). Thus at least a mark has been left where the growing center was, and another proof has been found that these conglomerations are really intravital germinative centers.

Finally, it may be asked how this peculiar conglomerative type of reproduction of spirochetes can be explained. In answering this, different factors must be considered.

First, it may be assumed that the reproductive activity of single spirochetes is rapid and that their motility in the tissues is relatively slow.

Second, the tissue resistance may in some way prevent the natural movement of spirochetes, for in fluid mediums massive conglomerations are not seen.

Third, some material of glue-like character may be produced by the spirochetes in earlier periods of growth or by the tissues themselves. Such a material would hold the spirochetes together. The lighter color of the inner parts of conglomerations in silver preparations could be explained in the same manner. But if parts of these inner centers are removed and stained for spirochetes, the organisms appear black, consequently the presence of a glue-like material seems improbable.

Fourth, the difference in argyrophilic affinity between the central and the peripheral parts of spirochetal conglomerations may be explained by a difference in density of the spirochetal mass. The inner parts may be much denser than the outer ones. For this reason the compact inner part may not take the silver salt solution as well as the outer zones. This explains the difference in color but not the specific type of growth in conglomerative masses. At present no explanation for this specific conglomerative type of reproduction can be offered.

#### SUMMARY

Spirochetal conglomerations seen in tissues in congenital syphilis and in dementia paralytica are compared with the similar appearing

spirochetal colonies in solid artificial culture mediums and with colonies seen in tissues of animal carriers (rats with leptospiras)

Areas of miliary necrosis in congenital syphilis and dementia paralytica are interpreted as the immediate consequence of these reproductive spirochetal conglomerations. One instance is cited in which miliary and submiliary necrosis in the pituitary in a case of congenital syphilis could be shown to be due to conglomerations of spirochetes.

Evidence has been given to indicate that these spirochetal conglomerations are reproductive centers.

Mr C. Graham Eddy made the excellent photomicrographs.

# RELATION OF THE ELASTIC TISSUE IN THE ROOT OF THE AORTA TO THE AORTIC VALVE

## INVOLVEMENT OF THIS TISSUE IN SYPHILIS

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The manner in which syphilis of the aorta may involve the cusps of the aortic valve and the characteristic deformities that ensue have been described by Saphir and Scott,<sup>1</sup> Bell and Clawson,<sup>2</sup> Martland<sup>3</sup> and others. The earlier literature was reviewed by Saphir and Scott.<sup>1</sup> It has been the general experience that the usual changes in the leaflets depend largely on alterations in the adjacent wall, especially at the cusps' highest and lateral points of attachment. While syphilitic valvulitis has been described,<sup>4</sup> its occurrence is relatively rare. The free margins and commissural attachments of the cusps are constantly involved, whereas the basal portions are almost always spared, even in advanced cases. Microscopically the cusps show marked fibrous overgrowth and hyalinization<sup>5</sup> rather than cellular infiltration or increased vascularity. Signs of inflammation, when they occur, are apt to be limited to the lateral borders.<sup>1</sup> Even if one assumed that the scarring of the cusps is the end result of previously active inflammation, it would be surprising indeed that so little vestige of the involvement persists, as the inflammatory character of the changes in the wall of the aorta is generally unmistakable. Moreover, it is extremely uncommon to observe changes in the leaflets of the valve without concomitant involvement of the root of the aorta. Thus, the weight of evidence seems to indicate that the cusps are secondarily and passively altered in much the same fashion as is the intima elsewhere. Primary specific inflammatory changes of syphilitic aortitis involving the intima directly are perhaps just as rare as similar changes in the leaflets of the aortic valve, the superior portions of which, at least, are but appendages of the inner layer of the aorta.

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1 Saphir, O, and Scott, R W. *Am J Path* **3** 527, 1927

2 Clawson, B J, and Bell, E T. *Arch Path* **4** 922, 1927

3 Martland, H S. *Am Heart J* **6** 1, 1930

4 Richter, A B. *Am J Path* **12** 129, 1936. Šikl, H, and Raska, K. *Časop lek česk* **76** 793, 1937, abstracted, *Arch Path* **26** 1072, 1938

5 MacCallum, W G. *A Textbook of Pathology*, ed 6, Philadelphia, W B Saunders Company, 1936, (a) p 701, (b) p 696

If these premises are conceded, it remains necessary to account for the frequent discrepancy between the degree of involvement of the root of the aorta and that of the aortic valve. The extent of valvular change does not appear to be directly proportional to any one feature of the lesion, not to the degree of dilatation nor to the amount of medial or intimal scarring nor to the intensity of the inflammation. This fact has been commented on by MacCallum,<sup>5b</sup> who stated "When the sinuses of Valsalva are affected, the aortic valves are likely to be involved and to undergo analogous changes, but every combination of aortic and valve involvement may occur, and sometimes the valves may remain perfectly delicate and competent when the nearby wall of the aorta is profoundly affected." Although the root of the aorta is one of the commonest and earliest sites of involvement, aortic insufficiency develops in only a relatively small number of cases of syphilitic aortitis (20.0 per cent, Call,<sup>6</sup> 36.5 per cent, Clawson and Bell,<sup>2</sup> 20.7 per cent, Welty<sup>7</sup>). Another finding which serves to emphasize this occasional disparity is the fact that syphilis of the sinuses of Valsalva may be severe enough to cause stenosis of the orifices of the coronary arteries without damaging the valve.

The appearance of the syphilitic lesion at the time of death can be deceptive, of course, and there is no quantitative way of measuring the intensity of the process. No very accurate information concerning the evolution of the changes can be obtained. It is possible that a rapidly developing and progressive aortitis damages the valve with greater facility than a more slowly evolving or an intermittent one. The possibility remains, however, that there is some variable component in the structure of the root of the aorta which may mediate involvement of the valve by rendering it more likely to occur in some instances and by preventing it in others. Variations in the amount of elastic tissue of the media beneath the valve might be accountable, since only those portions of the cusps beneath which elastic tissue is present are usually affected. The fact that the inflammatory changes frequently fail to extend deeply into the sinuses of Valsalva (Martland<sup>3</sup>) is also an indication that the process may end at the borderline of the media. That variations in the insertion of the media into the sinuses of Valsalva may occur might be suspected from the other minor malformations sometimes seen in this area. The cusps may vary in shape, size and number, and the orifices of the coronary arteries are frequently displaced or have accessory openings.

For these reasons the present study was undertaken in order to discover how much variation in the amount of elastic tissue may occur in

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6 Carr, J. G. *Am Heart J* 6:30, 1930

7 Welty, J. W. *Am J M Sc* 197:782, 1939

relation to the aortic valve in nonsyphilitic aortas, and whether it is of a sufficient magnitude to shield or to expose the cusps in the event of syphilitic inflammation. A comparable study was made on two groups of syphilitic hearts. One of these consisted of hearts in which the aortic valves were altered and incompetent, and the other, of hearts in which the valves were normal in spite of lesions in the nearby wall.

#### MATERIAL AND METHODS

Single sagittal, or profile, sections, 2 mm wide, were cut through the commissure between the right and the left aortic cusp (B N A terminology) of 73 nonsyphilitic adult hearts obtained at autopsies on the Third (New York University) Division of Bellevue Hospital. Hearts showing rheumatic or bacterial endocarditis or obvious congenital malformation were excluded. The blocks were cut so as to extend from 0.5 cm above the superior margin of the commissure proximally, to include a small portion of the left ventricular muscle. The position of the upper aspect of the commissure was indicated by a transverse knife cut into the intima. The tissue was fixed in solution of formaldehyde U S P (1:10), embedded in paraffin, sectioned at 10 microns and stained by the combined Weigert and Van Gieson method. An attempt was made in each case to obtain a section for microscopic study from the central portion of each block, approximating the midpoint of the commissure as closely as possible.

The commissure at the junction of the right and left cusps was chosen because its distance from the left ventricular musculature was readily measured. The membranous portion of the intraventricular septum is apt to lie proximally to the commissure of the right and posterior cusps, and the anterior leaflet of the mitral valve lies inferiorly to the commissure of the posterior and left cusps. The distance of these two commissures to some fixed point in the subvalvular region, therefore, is not easily obtained. However, for comparison, the commissure of the right and posterior cusps was studied in 22 hearts, and that of the posterior and left cusps in 15. The data from these are presented separately.

The stained preparations were projected at a magnification of 40 and the following measurements made: (1) the distance of the projection of elastic tissue beyond the highest point of the commissure proximally toward the left ventricle, (2) the distance between the highest point of the commissure and the point where the media first begins to narrow, (3) the distance from the highest point of the commissure to the beginning of the left ventricular musculature.

These measurements could be made with considerable accuracy in any given section. The only point about which any indecision occasionally arose concerned the end of the broad media, where it began to taper, since there was sometimes fraying of the elastic fibers in this vicinity. Repeated measurements usually varied within 0.1 cm. The method is also open to criticism on the ground that the processes of fixation probably alter the spatial arrangements somewhat, but all the material was treated in the same manner, and the results should be comparable with one another even though they do not represent the exact *in vivo* measurements. Other errors were introduced because there was no way of obtaining a section through the exact midpoint of the commissure and because obliquities in cutting could not be avoided. These errors are probably insignificant, however, since measurements made from multiple sections of a number of blocks varied within 0.15 cm.

A series of 45 syphilitic aortas which showed extensive involvement of the root of the aorta in all specimens but definite anatomic evidence of aortic insufficiency in 24 was prepared in a similar fashion. The anatomic criteria for the diagnosis of aortic insufficiency were separation of the cusps at their highest point of attachment, thickening of the free margins, endocardial pockets on the subvalvular portions of the left ventricular wall and dilatation and hypertrophy of the left ventricle. Specimens were not included in which the absence or presence of aortic insufficiency was not obvious.

The measurements obtained from the syphilitic group are open to more serious objections than those from the control group, since the lesion was destroying the very tissue under observation and the position of the commissure was subject to displacement. However, the end point of the elastica and the beginning point of the

TABLE 1—*Influence of Various Factors on Measurements of the Elastic Tissue at the Root of the Aorta in Relation to the Commissure Between the Right and Left Aortic Cusps*

Factor	Number in Group	Distance Between Commissure and End Point of Tapering Elastica	Distance Between Commissure and Limit of Broad Media	Distance Between Commissure and Left Ventricle
		Mean, Cm	Mean Cm	Mean, Cm
	73 (total group*)	0.74 (total group)	0.07 (total group)	1.20 (total group)
Sex				
Males	45	0.74	0.07	1.22
Females	27	0.73	0.06	1.15
Age				
25-49 years	23	0.71	0.07	1.16
50-69 years	36	0.75	0.06	1.21
70-85 years	13	0.74	0.08	1.22
Heart weight				
Less than 410 Gm	33	0.72	0.06	1.17
More than 410 Gm	33	0.75	0.10	1.20
Body length				
Less than 170 cm	34	0.71	0.07	1.15
More than 170 cm	29	0.74	0.09	1.23

\* Numerical differences in the totals of the various subdivisions are due to the fact that the data on some subjects were not recorded.

left ventricle could be determined accurately in most instances. Moreover, it was felt that by comparing the results from the syphilitic group with those from the nonsyphilitic group a satisfactory evaluation of the significance of changes in the former could be made, particularly with reference to the position of the commissure. It must be admitted that measurements on the end point of the broad media in the syphilitic group are at best only rough approximations.

## RESULTS

*Relation of Elastic Tissue to the Commissure Between the Right and Left Aortic Cusps in Nonsyphilitic Aortas*—As shown in table 1, the mean values obtained in 73 aortas indicate that the broad portion of the media ends  $0.07 \pm 0.009$  cm proximally to the highest point of attachment of the cusps, the media then tapering somewhat irregularly

to the apex of an elongated wedge-shaped extension  $0.74 \pm 0.010$  cm farther along. From this point to the beginning of the left ventricular endocardium, a mean distance of  $0.46 \pm 0.017$  cm, the wall is composed of dense fibrous tissue devoid of elastic fibers. In individual cases there was considerable variation, but in every instance some elastic tissue extended beyond the upper limit of the commissure. In one instance the media began to narrow 0.30 cm above the commissure, and in another it extended a scant 0.20 cm beyond it. In such vessels only a minimal amount of elastic tissue lay beneath the valve, so that changes limited to the elastica could hardly affect the commissure. On the other hand, in one instance the media did not begin to taper until 0.32 cm beyond the commissure and extended 1.15 cm before the elastic tissue terminated. In such a case the commissural attachment of the cusps was backed by

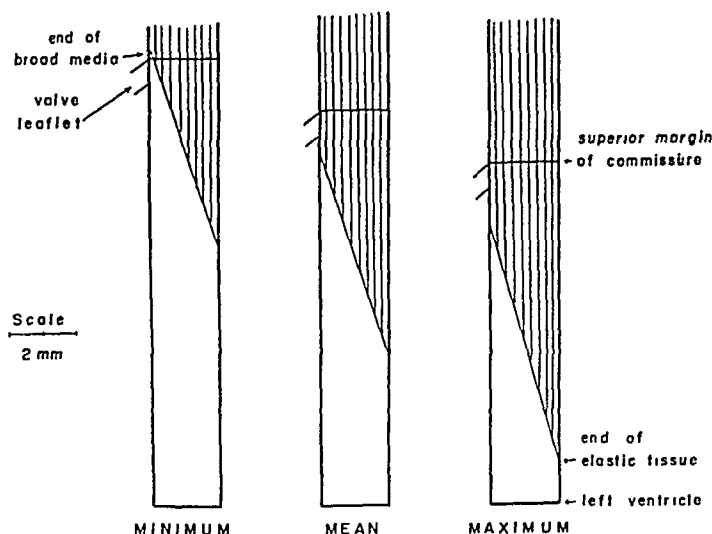


Chart 1—Relation of the elastic tissue at the root of the aorta to the commissure between the right and left cusps, with standard deviations

considerable underlying elastic tissue. Similarly, the distance between the end of the elastic tissue and the beginning of the left ventricle varied over a maximum range of 0.67 cm, so that the altered relations of the elastic tissue to the commissure were not based entirely on differences in the position of the latter.

The majority of the measurements fell close to the mean for each point, and symmetric distribution curves could be obtained in which the mean values closely approximated the modes. In chart 1 the relations of the various points under observation to each other are represented diagrammatically, and the maximum and the minimum range within the limits of the standard deviations are shown. It may be seen that even within these restricted borders the amount of medial elastic tissue adjoining the commissure may vary considerably. At the minimal values

only the tapering portion of the media lies beneath the cusps, whereas at the maximal values the broad media extends well beyond their point of origin

The results were analyzed to see if such factors as sex, body length, age and heart weight played a role in these variations. These factors influence the relative size of the heart and aorta. The measurements recorded here might depend on the size of these two structures. In females and short persons they are generally somewhat smaller than in males and tall persons. The aorta is also known to undergo progressive enlargement with age.<sup>8</sup> Further, the measurements of elastic tissue at the root of the aorta might be secondarily influenced by hypertrophy of the cardiac musculature.

When cases are grouped into these different categories (table 1), the mean values of the elastic tissue measurements are remarkably constant. Perhaps the distance between the upper limit of the commissure and the left ventricle is slightly less in females and subjects less than 170 cm long, but even this difference is not statistically significant. The relationship of the elastic tissue to the attachment of the aortic valve therefore depends on other individual idiosyncrasies and not on the relative size of the heart and aorta as influenced by the aforementioned factors.

*Relation of Elastic Tissue to the Commissure of the Right and Left Aortic Cusps in Syphilitic Aortas*—In table 2 are presented the mean values, with their probable errors, of similar measurements of elastic tissue in 24 syphilitic aortas showing definite evidence of aortic insufficiency and 21 showing no valvular lesion in spite of marked involvement of the sinuses of Valsalva. They are contrasted with the measurements on the control group of nonsyphilitic aortas.

In both syphilitic groups the upper limit of the commissure lies closer to the left ventricle than in the nonsyphilitic group. Although this displacement amounts to only 0.13 and 0.17 cm, respectively, both these differences are greater than four times the square roots of the sums of the squares of the respective probable errors and are therefore presumably significant. The exact mechanism of this displacement is not obvious, but it is not due to separation or other changes in the cusps, since it occurs to the same extent when the valves are not affected. Scott<sup>9</sup> also observed that in syphilis the cusps may be attached several millimeters below the normal site. Perhaps the stretching of the supra-

8 Kaufmann L. Zur Frage der Aorta angusta. Ein Beitrag zu den Normalmassen des Aortensystems, Jena, Gustav Fischer, 1919, Veröffentlichung d. Geb. d. Kriegs- u. Konstitutionspath. 1 (pt 2) 1, 1919.

9 Scott, R. W. Cardiovascular Syphilis, in Moulton, F. R. Syphilis, Publication 6, American Association for the Advancement of Science, Lancaster, Pa., The Science Press, 1938, pp. 118-122.



valvular portions of the aorta resulting from inflammatory changes and scarring is more effective on the outer, more pliable coats of the aortic wall than on the more resistant, although thinner, intimal layer

In any event this displacement alters the relations of the commissure with the elastic tissue of the media in both the incompetent and the competent valvular groups, so that in both the media appears to narrow at a higher point and to terminate a shorter distance beyond the commissure

If a correction is made for this altered position of the commissure, or if the distance between the end point of the elastic tissue and the left ventricle is taken, the nonsyphilitic group occupies a position intermediate between the two syphilitic ones. That is, the elastic tissue extends less beyond the true commissure and ends a greater distance

TABLE 2—*Extension of Elastic Tissue Beneath Left and Right Aortic Cusps in Syphilitic Aortas With and Without Aortic Insufficiency*

Group	Num ber in Group	Distance Between Commissure and End Point of Tapering Elastica Mean, Cm	Distance Between Commissure and Limit of Broad Media Mean, Cm	Distance Between Commissure and Left Ventricle Mean, Cm	Distance Between End of Elastica and Left Ventricle Mean Cm
Syphilitic aortas with aortic insufficiency	24	0.66 ± 0.031	0.016 ± 0.021	1.07 ± 0.029	0.41 ± 0.020
Syphilitic aortas without aortic insufficiency	21	0.45 ± 0.026	-0.08* ± 0.023	1.03 ± 0.022	0.58 ± 0.026
Nonsyphilitic aortas	73	0.74 ± 0.010	0.07 ± 0.009	1.20 ± 0.013	0.46 ± 0.017

\* The broad media ended distal to the upper border of the commissure

from the left ventricle in syphilitic patients without aortic valve insufficiency than in nonsyphilitic persons. On the other hand, in syphilitic patients with aortic insufficiency the elastic tissue extends farther beyond the original position of the commissure and ends at a point closer to the left ventricle than in nonsyphilitic persons.

When the data on the two syphilitic groups are compared (table 2), it is seen that the elastic tissue in the cases of syphilis with involvement of the aortic valve extended a mean distance of 0.21 cm farther beyond the commissure and ended 0.17 cm closer to the left ventricle than in the cases in which the aortic valve was not damaged. Although these differences are perhaps not impressive, they are of sufficient magnitude to be statistically significant. Moreover, in the latter group the media appeared to narrow 0.08 cm above the commissure, whereas in the group with aortic insufficiency the broad media reached the commissure. As already indicated, this measurement is not very reliable, because of the changes wrought by the syphilitic process, but it does support the impression that less elastic tissue is to be found beneath valves which are

unaltered by the lesion. The significance of these differences is best visualized in diagrammatic form (chart 2).

That the variations in the mean values are not due to the inclusion of a few unusual cases in each group is shown by the curves of the percental distribution of the distances between the end point of the elastic tissue and the left ventricle in chart 3. The curve for the distances in nonsyphilitic aortas (the solid line) is fairly symmetric, and its peak closely approximates the arithmetic mean. All the values for the syphilitic aortas lie within the extremes of the control series. In a major portion of those without aortic insufficiency, 81.0 per cent, the elastic tissue ends a greater distance from the left ventricle than the mean of the nonsyphilitic group. On the other hand, 70.8 per cent of the syphilitic aortas with insufficiency show elastic tissue which extends

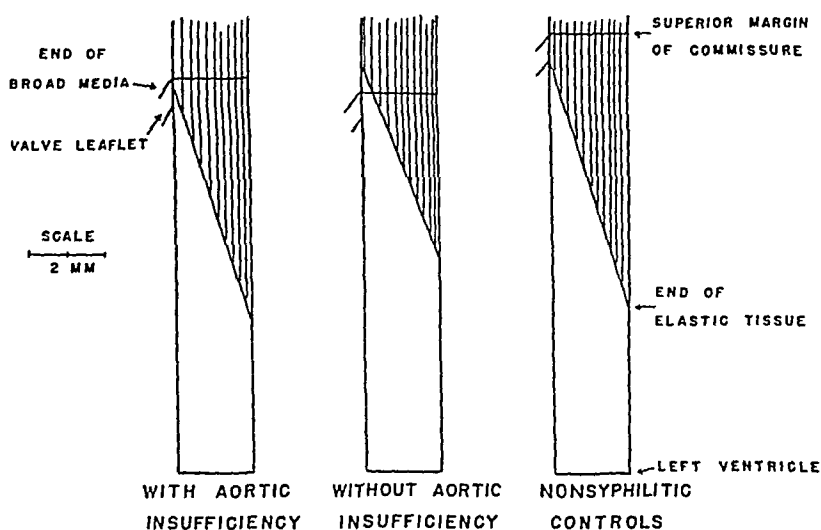


Chart 2—Mean measurements of the elastic tissue at the commissure between the right and left aortic cusps in syphilitic aortitis with and without aortic insufficiency.

closer to the left ventricle than that in the control group. The frequency distribution shows a greater difference between the two types of syphilitic aortas than do the mean values, for the greatest number of the values for the syphilitic aortas without insufficiency fall near 0.78 cm and the greatest number of those for syphilitic aortas with insufficiency lie near 0.33 cm, a discrepancy of 0.45 cm. The distribution shown by the curves suggests that when the syphilitic process involved aortas in which the elastic tissue ended farther than 0.46 cm from the left ventricle, aortic insufficiency was less apt to develop than when it attacked those in which the elastic tissue extended closer to the left ventricle than this.

*Amount of Elastic Tissue Beneath Each of the Three Aortic Valve Commissures and Its Relation to the Degree of Involvement of Each*

*Syphilitic Aortas with Aortic Insufficiency*—The extension of elastic tissue beneath each commissure varies in the same and different persons. Usually there is a significantly longer projection at the commissures between the right and posterior cusps and between the posterior and left cusps than at the one already discussed, namely, between the right and left cusps. If this is so, and if the amount of adjacent elastic tissue plays a role in determining the extent of involvement of the valve in syphilis of the root of the aorta, it might be expected that a correlation between the two is demonstrable.

In table 3 it may be seen that the elastic tissue at the attachments of the right and posterior cusps and at the attachments of the posterior and left cusps extends, on the average, 0.21 and 0.31 cm, respectively, farther beyond the highest point of the commissure toward the left ventricle than it does at the attachment between the right and left cusps.

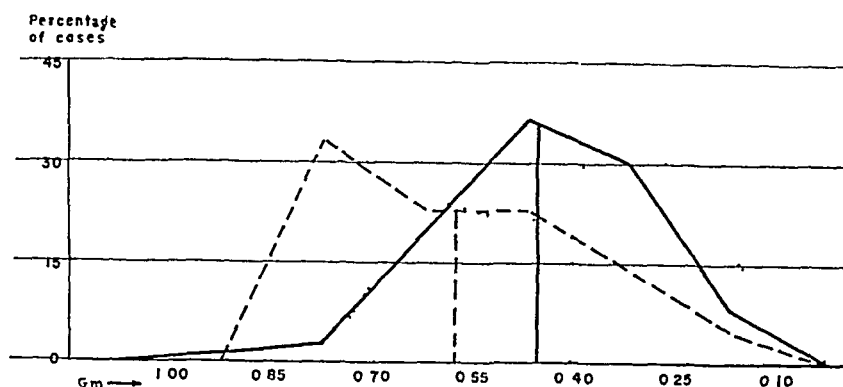


Chart 3—Percental distribution of distances between the end point of the elastic tissue of the aorta and the left ventricle in nonsyphilitic aortas and syphilitic aortas with and without insufficiency. The distances in nonsyphilitic aortas are represented by the solid line (73 cases, mean  $0.46 \pm 0.017$  cm), the distances in the syphilitic aortas with insufficiency by the dotted line (24 cases, mean  $0.41 \pm 0.020$  cm), and the distances in the syphilitic aortas without insufficiency by the dash line (21 cases, mean  $0.58 \pm 0.026$  cm). The mean value of each group is indicated by the corresponding vertical line.

Differences in a similar direction but of less magnitude and not statistically valid are shown between the end point of the broad media and the commissure in all three instances. One measurable feature of the syphilitic involvement at each of these three points, the degree of separation of the cusps, is compared. This was done by measuring the width of the gap between the two cusps at their closest point of approximation, by means of a hand lens, to the nearest 0.5 mm in a series of syphilitic aortas with aortic insufficiency. Differences in the number of observations are due to the fact that some commissures had been excised before this measurement was made. Although this is a crude method of estimating the degree of involvement at each commissure, the data do support

the concept that the distribution of elastic tissue is a factor in the development of commissural changes. The commissures possessing the largest amount of underlying elastic tissue showed the greatest mean degree of separation of the leaflets.

This was further borne out when the degree of involvement of each commissure in the same aorta was compared. The commissure with the least subjacent elastic tissue, that between the right and left cusps, showed the greatest degree of damage in only 20 per cent of 30 syphilitic

TABLE 3—*Extension of Elastic Tissue Beneath Each Commissure of the Aortic Valve, and Its Relation to the Degree of Involvement of Each in Syphilitic Aortitis with Insufficiency of the Aortic Valve*

Extension of elastic tissue proximal to commissure in nonsyphilitic aortas	Commissure at Attachment of					
	Left and Right Cusps		Right and Posterior Cusps		Posterior and Left Cusps	
	Aortas	Mean	Aortas	Mean	Aortas	Mean
1 Limit of broad media	73	0.07 ± 0.009 cm	22	0.11 ± 0.021 cm	15	0.16 ± 0.024 cm
2 End of tapering portion	73	0.74 ± 0.010 cm	22	0.95 ± 0.036 cm	15	1.05 ± 0.042 cm
Degree of separation of cusps at commissures in syphilitic aortas with involvement of valve	42	1.03 mm	55	1.15 mm	54	1.39 mm
Relative involvement of each of the three commissures in 30 syphilitic aortas *						
1 With greatest degree of involvement	6	20.0%	6	20.0%	13	43.4%
2 With least degree of involvement	9	30.0%	7	23.3%	2	6.7%

\* Those in which two or more commissures showed an equal amount of involvement are not included.

aortas and the slightest degree in 30 per cent. The commissure possessing the most elastic tissue, the one between the posterior and left cusps, was most affected in 43.4 per cent and least altered in 6.7 per cent. This evidence offers collateral support to the main thesis, that the extent of elastic tissue at the root of the aorta is of some importance in determining whether or not the valve will be liable to involvement in syphilitic aortitis.

#### COMMENT

The findings indicate that in the normal aorta the media extends proximally to about the upper margin of the commissure and then tapers to a variable length beyond it. Although the magnitude of variations in the extension of the media in this region is scant, it is of some significance because the only point of contact of the cusps with the aortic media is at their upper and lateral attachments.<sup>10</sup> In the central portions

of each sinus of Valsalva the media invariably terminates above the point of origin of the leaflet. Even a difference of a few millimeters in the extent of the media at the commissures can radically alter the relationship of the two. The measurements recorded here do not express completely the extent of this variation. In some hearts the angle formed by the adjacent aortic cusps at their lateral attachments is much more acute than in others. In such instances much more of the valvular attachment has aortic media beneath it. In some cases the media narrows abruptly at the upper limit of the commissure, and only a thin strand continues toward the left ventricle. In others the narrowing is much more gradual so that a fairly thick layer of media lies proximal to the upper limits of the cusps. Such variations alter the effective area beneath which the valve attachments have medial tissue.

It should be stressed that in every case some portion of the media extended at least to the highest point of attachment of the aortic valve. If involvement of the cusps depends secondarily on changes in the media, every aortic valve is potentially vulnerable to damage by syphilitic aortitis, at least to some extent. The severity and distribution of the inflammatory process thus remain the decisive factors in the development of aortic insufficiency. The findings reported here indicate merely that some valves may be much more readily involved than others, owing to variations in the preexisting structure of the valve ring. Whatever the exact mechanism of the changes in the leaflets may be, whether it is an extension of the inflammation from the wall or a process secondary to degenerative changes in the media which in turn are attributable to involvement of the adventitia or vasa vasorum, the fact cannot be ignored that only those portions of the valve beneath which the media is present are usually affected. Thickening of the free margin may well be caused by mechanical factors after aortic insufficiency has been established, according to Martland<sup>3</sup>. In those few instances in which active inflammation of the entire cusp is present and *Spirochaeta pallida* is demonstrable,<sup>4</sup> the development of aortic insufficiency is obviously more independent of the mode of attachment.

The significance of the differences observed in the elastica of the two syphilitic groups cannot be evaluated with certainty. These differences may be the consequence of variations in the syphilitic lesion and not express variations that existed before the inflammatory process was initiated. If this were so, however, it might be expected that the aortas showing the severest lesions, as indicated by the development of aortic insufficiency, would show the most marked destruction and reduction in elastic tissue. Yet more elastic tissue was found beneath commissures which were altered than beneath those which were not. If anything, the syphilitic process would tend to diminish and obscure the differences between the two syphilitic groups rather than to exaggerate them.

The possibility cannot be ruled out, however, that a certain degree of regeneration of elastic tissue occurs as the lesion progresses with ultimate extension of the elastic tissue beyond its original position. If this occurs at all, it might be more pronounced in the aorta with the severest involvement, namely, that with aortic insufficiency. The difference observed could thus be interpreted as the result of the lesion. The process elsewhere in the aorta, however, does not appear to be associated with any conspicuous degree of elastic tissue growth. There is a progressive loss of elastic tissue, with narrowing of the media, although the other coats are greatly thickened. Moreover, the range of measurements of elastic tissue in the syphilitic aortas was within that of the control series. If regeneration of elastic tissue was a factor in altering the measurements, in no instance did it extend closer to the left ventricle than it sometimes does in normal aortas. The weight of evidence is against the interpretation that secondary regeneration of elastic tissue alters the original distribution of this tissue to any appreciable degree.

#### SUMMARY

Measurements on the nonsyphilitic aorta reveal that the elastic tissue of the media at the root may project a variable distance proximal to the commissural attachments of the aortic valve cusps and that the lateral attachments of the cusps may show a great deal or very little of the medial coat in the wall of the underlying aorta. These variations do not appear to be related to the size of the aorta or heart and are not influenced by age, sex, body length or heart weight.

Since the lateral attachments of the aortic cusps are chiefly involved in the development of aortic insufficiency due to syphilis, it is pointed out that the degree of valvular damage may depend in part on the extension of the media in this area.

Measurements on the syphilitic aorta support this concept. The elastic tissue of the media is found to end more abruptly at the commissures in cases in which aortic insufficiency has failed to develop than in cases in which the valves are incompetent.

The three commissures in the normal aorta have different amounts of elastic tissue in their walls. Although the relative degrees of involvement of each of the three commissures varies in individual cases, in a series of cases of syphilitic aortitis with aortic insufficiency the average degree of involvement varied directly with the amount of intimal elastic tissue.

The highest point of attachment of the aortic cusps is found to be lower in the syphilitic aorta than in the normal one. This displacement is not related to changes in the leaflets themselves, since it is found when the cusps are still delicate and normally inserted.

# MYASTHENIA GRAVIS AND THE THYMUS GLAND

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Recent papers, particularly those of Norris,<sup>1</sup> have again drawn attention to the relation between myasthenia gravis and the thymus. They have pointed out that only 82 autopsies on the disease are recorded in the literature and that in 37 of these a lesion of the thymus was found to be a prominent anatomic feature. Norris emphasized the importance of recording the pathologic observations in all investigated cases of myasthenia gravis.

The association of a tumor of the thymus with myasthenia gravis was first described by Weigert,<sup>2</sup> in 1901. He considered the tumor a lymphosarcoma and looked on the lymphorhages in the voluntary muscles as metastases. Buzzard<sup>3</sup> contested this view, demonstrating lymphorhages without apparent involvement of the thymus, and after Marburg<sup>4</sup> lymphorhages were increasingly regarded as signs of a reactive process in degenerating muscle. Hart<sup>5</sup> suggested that the "tumors" of the thymus were merely different degrees of hyperplasia of the epithelial and lymphoid cells of the gland.

Bell<sup>6</sup> and Norris<sup>1a</sup> reviewed series of cases of myasthenia with regard to thymic involvement, finding it in at least 50 per cent of adequately reported cases. Norris stated that "pathologic changes may be found in the thymus in cases of myasthenia gravis in direct ratio to the care with which they are sought." Thus, none were reported prior to 1901. Starr<sup>7</sup> found them in 28 per cent of a series of 250 cases collected from the literature, Bell<sup>6</sup> in approximately 50 per cent of his 56 cases and Norris<sup>1</sup> in 50 per cent of his further cases. Bell stressed the specific character of the changes in the thymus—a reversion to a fetal type of structure produced by the disproportionate hyperplasia of the epithelial elements, which are relatively inconspicuous in the adult.

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From the Department of Pathology of the Johns Hopkins University

1 Norris, E. H. (a) *Am J Cancer* **27** 421, 1936, (b) **30** 300, 1937

2 Weigert, C. *Neurol Centralbl* **20** 597, 1901. Laquer, L. *ibid* **20** 594, 1901

3 Buzzard, E. F. *Brain* **28** 438, 1905

4 Marburg, O. *Ztschr f Heilk* **28** 111, 1907

5 Hart, C. *Virchows Arch f path anat* **220** 185, 1915

6 Bell, E. T. *J Nerv & Ment Dis* **45** 130, 1917

7 Starr, M. A. *J Nerv & Ment Dis* **39** 721, 1912

gland—and pointed out that such an appearance is found almost exclusively in tumors associated with myasthenia gravis. Norris described the condition as a benign tumor in half the cases and as persistent enlargement in half, but put forward the view that this division is artificial and that both classes represent degrees of hyperplasia, extreme and moderate, respectively.

Obiditsch<sup>8</sup> studied histologically a series of 9 thymus tumors and claimed that those composed predominantly of small round cells were more often associated with symptoms of myasthenia than were epithelial and malignant types.

Meggendorfer<sup>9</sup> and Meister<sup>10</sup> reported cases of a metastasizing growth of the thymus associated with myasthenia gravis, but such cases are extremely rare, thus Meggendorfer's case was at that time the only one of 60 recorded cases of malignant tumor of the thymus in which the tumor was associated with myasthenia.

Schumacher and Roth<sup>11</sup> and Harer<sup>12</sup> reported cases in which improvement of myasthenia followed extirpation of a tumor of the thymus, the operation in the first case having apparently been performed by Sauerbruch. Beretvas<sup>13</sup> had no success in the treatment of the disease by irradiation of the thymus, but Stein<sup>14</sup> reported improvement obtained by this means.

Recently Adler<sup>15</sup> claimed that he had produced myasthenia-like symptoms of exhaustion in dogs by transplanting thymus tissue or by injecting a watery extract of juvenile thymus and that the animals' symptoms were rapidly relieved by injections of prostigmine. This work is as yet unconfirmed, and the account is lacking in detail. Nevin<sup>16</sup> claimed that injections of a commercial extract of thymus had produced aggravation of symptoms in some cases of myasthenia gravis and pointed out that administration of thyroid extract is followed by a temporary exacerbation which may be due to stimulation of the thymus.

Lievre<sup>17</sup> summarized the evidence for the importance of the thymus in myasthenia gravis thus: 1. A tumor or hypertrophy of the thymus

8 Obiditsch, R. A. *Virchows Arch f path Anat* **30** 319, 1937.

9 Meggendorfer, F. *Ann d städt allg Krankenh zu Munchen* **13** 116 1908.

10 Meister, M. *Klin Wchnschr* **15** 1389, 1936.

11 Schumacher and Roth. *Mitt a d Grenzgeb d Med u Chir* **25** 746, 1913.

12 von Harer<sup>er</sup>, H. *Arch f klin Chir* **109** 193, 1918.

13 Beretvas, L. *Riforma med* **41** 771, 1925.

14 Stern, R. *Wien klin Wchnschr* **50** 321, 1937.

15 Adler, M. *Arch f klin Chir* **189** 579, 1937.

16 Nevin, S. *J Neurol & Psychiat* **1** 120, 1938.

17 Lievre, J. A. *Presse med* **44** 991, 1936.



is frequently found in myasthenic patients, and the tumor appears to be a specific lesion. Microscopic tumors may easily be missed at autopsy. 2 The variability of the lesion (hyperplasia, circumscribed tumor) of the thymus applies also to other endocrine disorders. 3 While the diffuse hyperplasia of the gland could legitimately be considered a part of the general proliferation of lymphoid tissue common in this disease, such an explanation seems unlikely in the case of a circumscribed tumor. Lièvre concluded that there is a relation between the thymus gland and myasthenia gravis and that the thymic condition is probably not a non-specific reaction but either causal or concomitant. He recommended roentgen investigation, followed by irradiation or surgical removal.

#### REVIEW OF FIVE CASES

The pathologic observations in the 5 cases of myasthenia gravis encountered in 16,300 autopsies at the Johns Hopkins Hospital will now be summarized, only positive evidence being given.

CASE 1—A white man aged 35 had a twelve month history of increasing weakness of the muscles of mastication and respiration. His death was due to respiratory failure.

The autopsy report was "A clinical history of myasthenia gravis, a tumor nodule in the region of the thymus, a stone in the pelvis of the left kidney, hydronephrosis, pulmonary edema, localized bronchopneumonia, old tuberculosis of the bronchial nodes."

The thymus was fatty and not noticeably enlarged but showed two strips of tissue extending about one third of the way down over the parietal pericardium. Embedded in the midportion of the left lobe was a small firm nodule, measuring 2 by 1.5 by 1 cm, well encapsulated, elliptic and sharply defined from the surrounding normal thymus tissue.

On section this was grayish red and showed small blood-filled spaces, it had a gross appearance similar to the surrounding thymus tissue but was more compact in its arrangement and contrasted sharply with neighboring lymph nodes, which were pigmented and in some instances caseous.

Histologically, the surrounding tissue, stretched around the tumor in isolated patches embedded in loose areolar tissue, it showed the normal structure of the thymus gland with well marked Hassall's corpuscles. The tumor itself was well defined and trabeculated by a connective tissue capsule, it was vascular and was composed mainly of lymphoid cells, with some hyperplasia of the epithelial elements, especially at the periphery (fig 1 *A* and *B*).

There were no Hassall's corpuscles in the tumor, and the epithelial cells showed no signs of the compression or tension which flattens them in the normal thymus, but had an indistinct cell body and a plump, vesicular nucleus. They extended into the substance of the tumor in a network quite distinct from the masses of lymphoid cells.

Lymphorrhages were present in the voluntary muscles but were rare, being found in only 1 of 18 sections. The thyroid gland contained a distinct excess of colloid, and many alveoli were lined with low, flat epithelium. The other endocrine glands showed no abnormality.

CASE 2—A white man aged 45 had an eight month history of increasing weakness of the muscles of mastication, phonation, deglutition and ocular movement. Death occurred from respiratory failure.

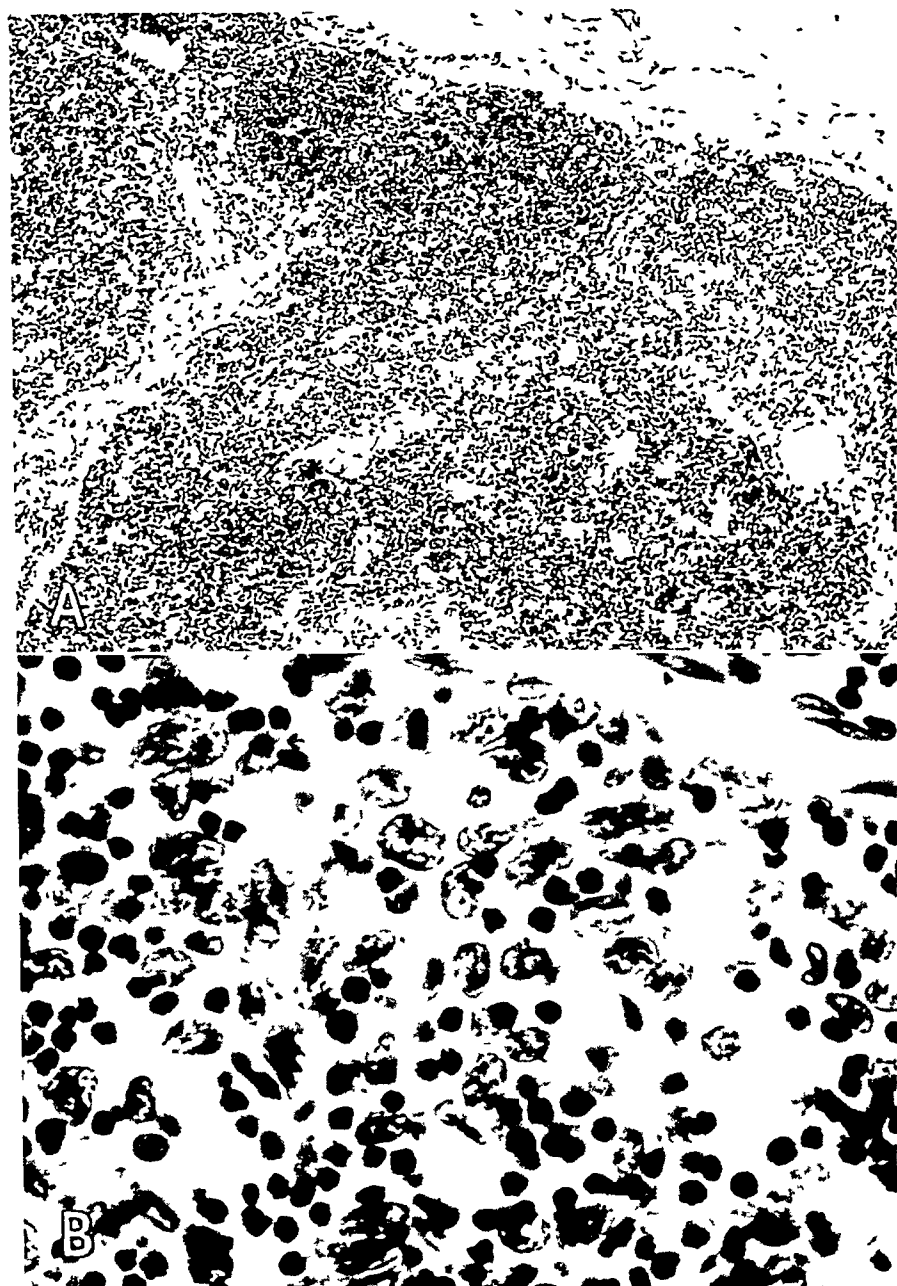


Fig 1 (case 1) —A, thymus tumor,  $\times 90$  B, same tumor, showing epithelial cells  $\times 670$

The diagnosis at autopsy was "myasthenia gravis persistent thymus, bronchitis, bronchopneumonia, fibrous nodule in the thyroid, fibrous orchitis, hemangioma of the liver, lymphangioma of the spleen, accessory spleen."

There was a bilobed thymus, each lobe measuring 7 by 3 by 1.5 cm, the gland weighing 60 Gm in all. There was no localized tumor, and histologically the gland consisted of scattered small foci of lymphoid cells embedded in areolar tissue. Some of these foci contained recognizable germinal centers, others, Hassall's corpuscles, which were sometimes calcified. In several places large epithelial cells which inconspicuously surrounded these foci had proliferated, forming small sheets of clear cells, with prominent nuclei, which looked very much like stratified squamous epithelium.

No lymphorrhages were found in the many sections of muscle taken, the thyroid gland was normal except for a hyaline fibrous nodule similar to that seen in the scarred testicles. There was no other abnormality of the endocrine system.

The lungs disclosed, in addition to acute lobular pneumonia, numerous collections of lymphoid cells, particularly around the small blood vessels, and sometimes these showed recognizable germinal centers.

CASE 3—A white woman aged 27 suffered for four years with slowly progressive weakness of the ocular muscles and of the muscles of mastication and deglutition. She improved when treated with ephedrine, but finally respiratory failure responded only temporarily to prostigmine and was fatal.

The diagnosis at autopsy was "myasthenia gravis, lobular pneumonia, persistent thymus, focal necroses in the adrenal cortex."

The thymus was a rather flat sheet of tissue, measuring 6 by 6 by 1 cm and weighing 30 Gm. Histologically the gland was uniformly normal, being composed of small round cells with well marked Hassall's corpuscles and with only an occasional suggestion of any differentiation into peripheral and central zones.

There was some scattered necrosis in the fasciculate and glomerular zones of the adrenal cortex in both glands, the cells being replaced by a pink formless material.

No lymphorrhages were found, and the thyroid gland and other endocrine organs were normal.

CASE 4—A white man aged 63 said that for six months he had had increasing muscular weakness, with improvement on administration of prostigmine, but that gradually this treatment lost effect. There was terminal respiratory distress, and the patient died despite the administration of prostigmine and treatment in the Drinker respirator.

The diagnosis at autopsy was "myasthenia gravis, lymphocytic infiltration in the muscles, a scar with fibrous adhesions at the apex of the upper lobe of the right lung, pulmonary edema, peculiar fibrosis of the leaflets of the mitral valve, fatty infiltration of the liver, moderate generalized arteriosclerosis with arteriosclerotic changes in the kidneys, calcified prostatic concretions, atrophy and scarring of the testis."

The thymus was not identified. Lymphorrhages were frequent in the voluntary muscles but only rarely were they perivascular. The thyroid gland was normal, and the only other abnormalities were those already summarized.

CASE 5—A colored man aged 34 had marked exophthalmos of long standing and a doubtful history of symptoms of exophthalmic goiter. For eight months he had noted increasing weakness of the musculature of the head and neck. He was maintained for two months on prostigmine, which he took irregularly. He died in respiratory failure despite the use of prostigmine and the Drinker respirator.

The diagnosis at autopsy was "myasthenia gravis, a tumor of the thymus with remnants of normal thymus tissue, widespread small areas of lymphorrhagia in the muscles and elsewhere, hyperplasia of lymphoid nodules, especially in the pharynx and larynx, colloid goiter, lobular pneumonia"

The thymus weighed 70 Gm and was the size and shape of a bantam's egg (fig 2) Most of the gland was taken up by a large tumor, 5 by 3 by 6 cm, with a little normal thymus tissue at the lower pole. The tumor was divided by fine bands of fibrous tissue into lobules of varying size. These contained a mixture of small lymphoid cells and a network of large, ill defined, pale epithelial cells with vesicular nuclei (fig 3A). The proportion of these two kinds of cells varied considerably, and in some areas the "epithelial" elements formed solid blocks of tissue (fig 3B), while in other lobules they were diffusely scattered among the round cells.



Fig 2 (case 5) —Thymus tumor

The lower pole of the tumor was covered by a thin layer of thymus tissue, which was pink in the gross as opposed to the creamy tumor, and was histologically similar to normal thymus though relatively poor in Hassall's corpuscles.

The lymphorrhages were widespread, were perivascular and were found in the adrenal and in the epiglottis, as well as in the pharyngeal, ocular, intercostal and sternocleidomastoid muscles and the diaphragm (fig 3C). In the ocular muscles there were in addition, scattered areas of atrophy, where the muscle fibers had almost disappeared, leaving what appeared to be an empty fibrous framework (fig 3D). The general lymphoid hyperplasia spared the abdomen and affected only the regions above the diaphragm. The tonsils were huge and hyperplastic and the pharyngeal and laryngeal follicles extended continuously down into the piriform sinuses and larynx. There was a well marked colloid goiter, which also showed great lymphoid infiltration and some germinal centers.

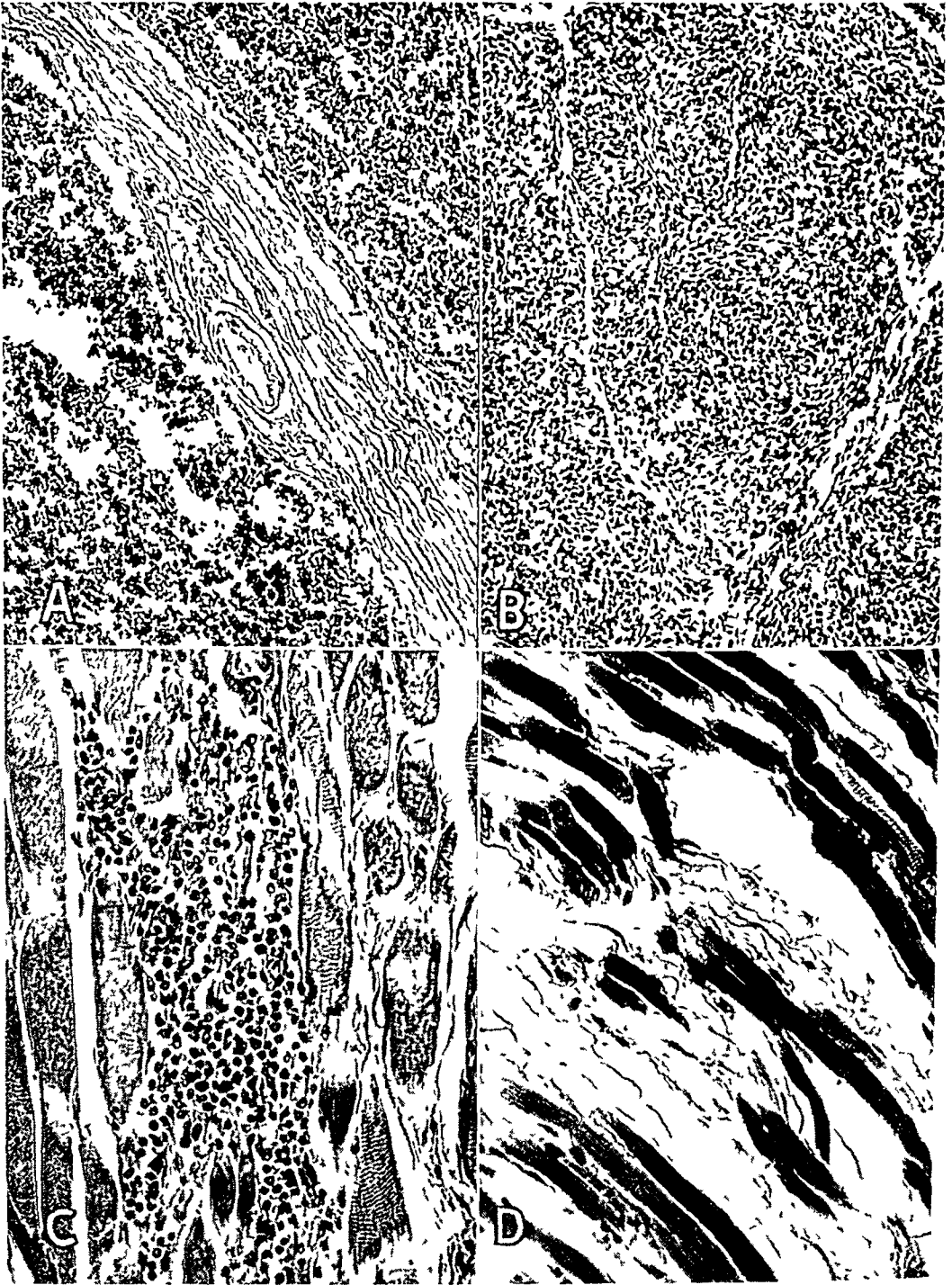


Fig 3 (case 5) — *A*, thymus showing areas consisting predominantly of lymphoid and epithelial cells, respectively,  $\times 100$  *B*, area of tumor composed of solid blocks of epithelial cells,  $\times 100$  *C*, typical lymphorrhage from the diaphragm,  $\times 200$  *D*, focal areas of atrophy in ocular muscle,  $\times 200$

## COMMENT

Of the 5 cases of myasthenia gravis reported here, 2 showed an encapsulated tumor of the thymus gland with remnants of normal thymus outside the capsule, 2 showed a persistent thymus gland, with well marked peripheral epithelial hyperplasia in 1 case, and in 1 case the thymus was not identified

This brings the number of autopsies in such cases reported in the literature to 87, in 41 of which the lesions in the thymus were found to be a prominent anatomic feature

It is interesting that in the present 5 cases abnormalities in the thymus were more common pathologically (4 cases) than the characteristic lymphorrhages, which are the accepted criteria of a pathologic diagnosis of the disease, though often difficult to find in clinically indisputable cases

As regards the role of the thymic condition in the genesis of the disease little can be added. Injection of extracts prepared from such tumors might assist in the elucidation of the problem. In the meantime it seems that careful roentgenologic examination of myasthenic patients is indicated in an effort to diagnose thymic involvement and that irradiation and surgical removal should be essayed in a disease which, despite recent therapeutic advances, is otherwise so lethal in its effects

## SUMMARY

The literature dealing with the relation of the thymus to myasthenia gravis is reviewed, and 5 further cases in which autopsies were done are described. In 2 cases an encapsulated tumor of the thymus was found associated with remnants of normal thymus, in 2, a persistent thymus was observed, with marked peripheral epithelial hyperplasia in 1 case and in 1 case the thymus was not identified

This brings the number of reported cases of myasthenia gravis in which autopsy was done to 87, in 41 of which distinct anatomic lesions of the thymus were found

It is suggested that patients with myasthenia gravis be subjected to a careful roentgen investigation in an effort to diagnose thymic involvement, and that irradiation and surgical removal be essayed more often in the treatment of this disease with so unfavorable a prognosis

# ADDITIONAL OBSERVATIONS ON POSITIVE AND NEGATIVE CHEMOTAXIS

EXPERIMENTS WITH A MYXOMYCETE

DALE REX COMAN, M D

PHILADELPHIA

In a recent paper <sup>1</sup> it was reported that negative chemotaxis could be induced in polymorphonuclear leukocytes by certain chemical substances (silicates) and, in some experiments, by a strain of hemolytic streptococci. Negative chemotaxis, i e the reaction through which leukocytes are caused to move away from bacteria or other bodies, may well be an important factor in infection, one tending to hinder recovery. This subject has not received the attention accorded to the opposite phenomenon, positive chemotaxis and requires further investigation. But before continuing the studies on negative chemotaxis in leukocytes it seemed desirable to obtain broader knowledge as to the occurrence of this reaction in living cells generally. Through observations on a different and perhaps more primitive type of organism it was hoped that negative and positive chemotaxis would appear in better perspective.

The plasmodium of *Physarum polycephalum*, a myxomycete <sup>2</sup> (slime mold), was selected for the present study. It shows the same type of locomotion (i e, ameoboid) as the leukocytes and, like the latter, displays both positive and negative chemotaxis. This organism proved suitable for some experiments for which the leukocyte on account of its small size, vulnerability and environmental requirements, is less well adapted. Experiments were planned with the myxomycete to answer the following questions. What types of chemical substances induce, respectively, positive and negative chemotaxis? Is the positive or negative reaction dependent on the nature of the substance tested or, as has been proposed <sup>3</sup>

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This investigation was aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association

1 McCutcheon, M , Coman, D R , and Dixon, H M Arch Path **27** 61, 1939

2 It is interesting to recall that myxomycetes were used by Stahl (Bot Ztg **42** 145 and 161, 1884) in some of the earliest experiments in chemotaxis

3 Wells, H G Chemical Pathology, ed 5, Philadelphia, W B Saunders Company, 1925

on the concentration? How does a cell or organism act when it alters its response from positive to negative? Is its behavior qualitatively different, or is a negatively reacting cell the mirror image of one reacting positively?

#### MATERIAL

The myxomycete, or slime mold, is a naked mass of protoplasm, a syncytium which occurs naturally in such an environment as the bark of a tree, where it seeks food by an ameboid type of locomotion and where it may attain a size of several centimeters. Its large size permits experimental observations to be made with the naked eye, or minute pieces may be cut off and observed with the microscope.<sup>4</sup> Both these methods have been employed in the present experiments. The culture of *Physarium polycephalum* was supplied by Prof. W. Sclafiz, of the department of botany. This is an orange-colored organism which grows readily in the laboratory, cultures being maintained on wet filter paper sprinkled with dry oatmeal.

#### METHOD

Observations with the naked eye were made on plasmodia placed in Petri dishes containing 2 per cent agar in distilled water. Disks were cut out of the gelled agar, one in the center and one or more at the periphery. Into the central well thus formed was poured agar containing oatmeal, and into the other wells, agar containing the substance to be tested for its chemotactic effect. The mold was planted on the central disk, and the direction of migration in relation to the disks of test substance was noted during the following eighteen hours.

The different types of reaction of the mold to test substances are shown in figures 1 to 3. Figure 1 illustrates an indifferent reaction (absence of chemotaxis). The mold has extended in all directions and has evidently been neither attracted nor repelled by the test substance in the disks near the edge of the plate. Figure 2 illustrates positive chemotaxis. The organism has migrated from the center to both disks containing the test substance. Figure 3 illustrates negative chemotaxis and is the opposite of figure 2, since, instead of moving toward the disks containing the test substance, the mold has avoided them.

#### CHEMOTACTIC EFFECTS OF SUGARS, ACIDS AND ALKALIS

By the method just outlined, the chemotactic effects of diffusible substances are readily tested. It is these substances—sugars, acids, alkalis, salts, present everywhere, taking part in all sorts of cellular processes—that, because of their rapid diffusibility, are especially difficult to test with leukocytes. The diffusion of acids and alkalis through the agar jelly may be observed by adding an indicator, such as bromthymol blue, to the agar.

A summary of results obtained with these diffusible substances is given in the table. Dextrose induced positive chemotaxis over a wide

<sup>4</sup> Such small pieces in appearance and response are quite comparable to leukocytes or other naked cells endowed with ameboid movement. The suitability of the slime mold for other types of investigation is obvious. For instance, in studies of injury and repair one might find in this a particularly favorable material.



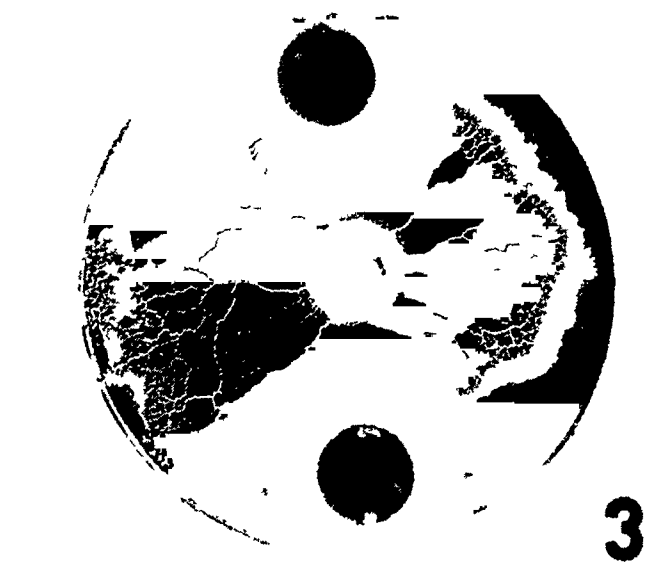
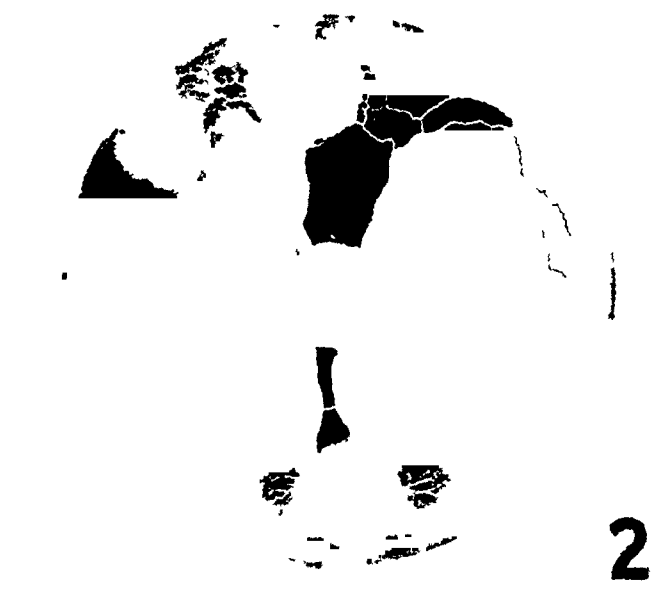
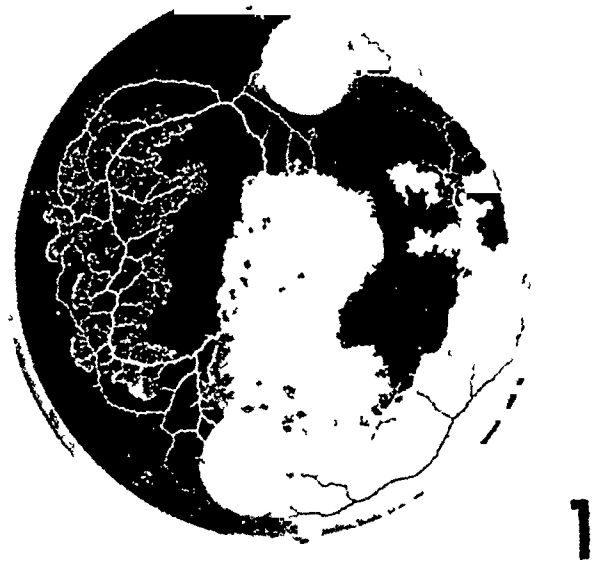
## EXPLANATION OF FIGURES 1, 2 AND 3

Photographs are shown of myxomycetes on agar in 100 mm Petri dishes. In each plate are three black circles, the central one of which represents a disk containing nutrient substance, on which the mold was planted. The peripheral disks contain the substance to be tested for chemotactic effect. The plasmodium appears as irregular gray sheets and filaments.

Figure 1 shows indifferent chemotaxis. The substance tested was sodium chloride. The mold has moved in all directions and shows no orientation to the test substance.

Figure 2 shows positive chemotaxis. The test substance was dextrose. The plasmodium has moved from its original central position to both disks containing the test substance and is seen nearly to surround these disks.

Figure 3 shows negative chemotaxis to a silicate (Lloyd's reagent). The plasmodium, in moving from the center to the periphery of the plate, has avoided the two disks containing the test substance.



Figures 1, 2 and 3

range of concentrations, from hundredth-molar to molar. The reaction was positive in 42 of 44 plates. Saccharose, it is interesting to observe, caused no chemotactic response in any of the concentrations used. The mold moved toward the sugar in 13 plates and away from it in 17, but generally the displacement toward or away from the test substance was slight, and therefore it is concluded that this organism is indifferent to saccharose. Thus the mold is able to discriminate between two closely related sugars. The chemotactic effect of different sugars apparently varies with the species of slime mold.<sup>5</sup>

The effect of hydrogen and hydroxyl ions was tested, using hydrochloric acid, sulfuric acid, acetic acid and sodium hydroxide. As seen in the table, all these substances in concentrations of tenth molar or

*Chemotactic Reactions of Myxomycetes to Substances Diffusing Through Agar Jelly Contained in Petri Dishes<sup>4</sup>*

	Preparations of Myxomycetes Reacting to Given Molar Concentration of Substance																	
	0.01		0.02		0.05		0.1		0.2		0.5		1.0		2.0		4.0	
Test substance	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Dextrose	4	0	5	0	5	0			8	2	10	0	10	0				
Sucrose									2	3	2	5	6	4	2	3	1	4
Hydrochloric acid	2	3					0	19										
Sulfuric acid	3	3					0	6			0	6	0	6				
Acetic acid	4	4					0	6			0	6	0	6				
Sodium hydroxide	2	3					1	5	0	10	0	12						
Sodium chloride							7	7										

\* Results were recorded about eighteen hours after planting. A positive (+) reaction indicates movement toward the test substance, a negative (—) reaction, movement away from it. The mold was attracted by dextrose in all the concentrations used; it was indifferent to sucrose. Acids and alkalis in concentrations of tenth molar or greater produced negative chemotaxis.

above produced negative chemotaxis, lower concentrations had no effect. Combining the results obtained with the various acids, one finds that the mold was repelled in 55 plates and attracted in none. A similar effect was obtained with sodium hydroxide, the chemotaxis in 1 plate being positive and that in 27 negative, with concentrations of tenth molar or higher. That these results were probably due to hydrogen and hydroxyl ions, respectively, rather than to sodium or chlorine ions or to osmotic effects is seen in the indifferent response to tenth molar sodium chloride in 7 plates; chemotaxis was weakly positive and in 7 weakly negative.

It is concluded from these experiments that both hydrogen and hydroxyl ions when in adequate concentration produce negative chemotaxis in *Physarum polycephalum*. This result is in general agreement with those reported by Strange<sup>6</sup> and Emoto<sup>5</sup> with other species of

<sup>5</sup> Emoto, Y. Proc. Imp. Acad., Tokyo 8: 460, 1932.

<sup>6</sup> Strange, B. Bot. Ztg. 48: 107, 1890.

slime mold and by Jochims<sup>7</sup> with leukocytes. These workers, however, reported positive chemotaxis to certain weakly acid solutions, a result not duplicated in the present experiments. The effect of hydrogen and hydronium ions may be summarized by saying that both cause repulsion, the least repelling concentration for some organisms being somewhat on the acid side of neutrality.

In addition to the repelling effect of high concentrations of hydrogen and of hydronium ions on both myxomycetes and leukocytes, other points of similarity in the responses of the two types of organisms have been observed. Thus both are repelled by a silicate (Lloyd's reagent).<sup>1</sup> Also, like myxomycetes, leukocytes are reported to be attracted by certain sugars.<sup>8</sup> Whether they react similarly to other substances must be decided by further experiments.

#### EXPERIMENTS WITH THE MICROSCOPE

The slime mold is excellently adapted to microscopic observation and much information has been obtained by low power magnification. A small piece of the mold, about 0.5 mm. long, is snipped off with fine scissors and placed in a thin film of water on a glass slide. The slide is inverted over a small dish to form a moist chamber.

As seen under low power magnification after a period of quiet streaming of the protoplasm begins. The streaming has the remarkable characteristic of rhythmicity: the protoplasm flows for many seconds—often 40 to 60—in one direction, and then reverses and flows for approximately the same time in the opposite direction. (For detailed study of protoplasmic streaming see Vouk<sup>9</sup> and Seifriz<sup>10</sup>.) As locomotion develops, the rhythm of protoplasmic streaming alters, the flow toward the advancing pseudopod continues longer than the reflux in the opposite direction.

If an attracting substance, such as a fragment of oatmeal, is placed a few millimeters from the mold, a broad pseudopod is thrust out toward it. The protoplasm in the pseudopod appears to become more liquid, as judged by the turbulent motion of the protoplasmic granules. From the front of the pseudopod are thrust out clear blister-like extensions of protoplasm which, from the rapidity of their formation and flow, appear to have relatively low viscosity. In a few seconds the granular cytoplasm flows into the clear processes, and thus the pseudopod rapidly advances. The organism now assumes a triangular shape, with the broad

7 Jochims, J. *Arch. f. d. ges. Physiol.* **216** 611, 1927.

8 Chambers, R., and Grand, C. G. *J. Cell & Comp. Physiol.* **8** 1, 1936.

9 Vouk, V. *Denkschr. d. k. Akad. d. Wissensch. Math.-naturw. Kl.* **88** 653, 1913, cited by Kisser and Metzner. *Tabulae biol.* **4** 471, 1927.

10 Seifriz, W. *Science* **88** 21, 1938.

pseudopod in advance and a narrow tail behind. The organism in this state of activity looks much like a macrophage.

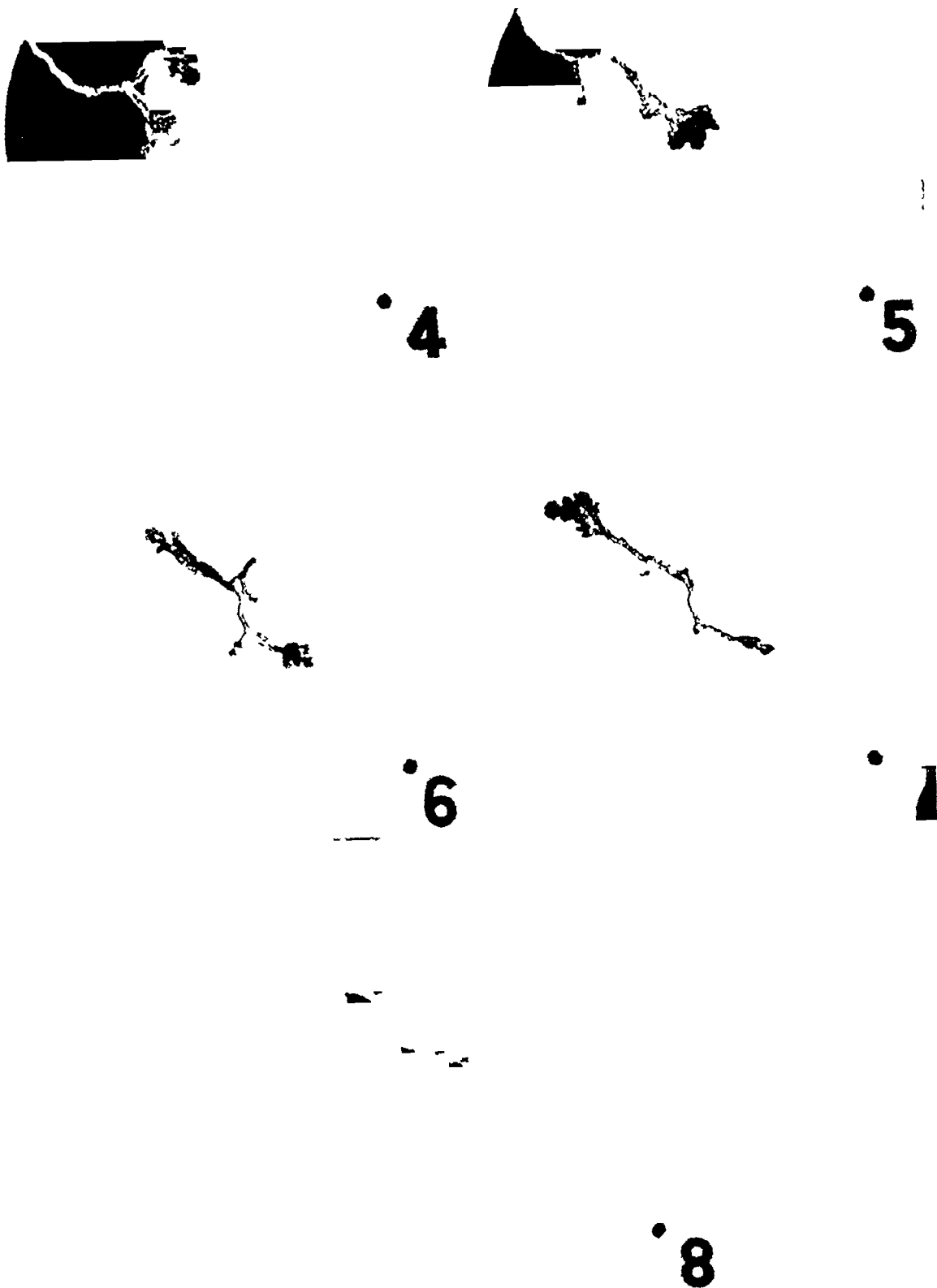
The positive and negative responses of this "cell" to chemical stimuli may be illustrated by a representative experiment. The appearance of the mold displaying positive chemotaxis is shown in figures 4 and 5. The source of attraction is just outside the photographic field and is indicated by the white dot. In figure 4 the organism is advancing into the microscopic field and shows two pseudopods at its anterior end, three minutes later, one of these pseudopods has retracted as the mold continues its advance. During all this time, of course, the rhythmic protoplasmic streaming goes on, being of longer duration in the direction of the attracting substance.

After the latter photograph was taken, the attracting substance (oatmeal) was removed, and a repelling substance, a silicate, was placed in the same position. Figures 6, 7 and 8 show the subsequent behavior of the organism. Within three minutes the advancing pseudopod seen in the preceding photograph has shrunk, and a new pseudopod has developed at the opposite end of the organism. In figure 7 the original pseudopod has nearly disappeared, while the pseudopod at the opposite end has broadened, and the organism has begun to move away from the repelling substance. In the last photograph (fig. 8) the mold is seen leaving the field.

Thus change from positive chemotaxis to negative has brought about reversal of polarity, the former "head" has become "tail," the former "tail" has become "head." The organism has not turned around but has "gone into reverse."

At the same time reorientation of the organism has occurred in other respects. It is now the end farthest from the negatively chemotactic substance that appears liquid, and at this end form clear blister-like extensions of the pseudopod, into which the granular protoplasm flows. At the end nearest the source of repulsion the changes are of opposite character. As this end contracts it becomes less fluid, i. e., protoplasmic inclusions move less turbulently, as if the substance were highly viscous. The duration of the phases of rhythmic streaming alters so that the flow is now longer in a direction away from the repelling substance.

Thus the organism when reacting negatively is the mirror image of itself when reacting positively. The difference is simply that between minus and plus, there is no qualitative difference. If the observer saw the organism but not the source of the substance to which it reacts, he would be unable to say whether chemotaxis was positive or negative, since the appearance of the organism and the rhythm of streaming are the same in both cases.



#### EXPLANATION OF FIGURES 4 TO 8

These are low power photomicrographs of a small piece of plasmodium, taken at intervals of three minutes, to show its appearance while it is displaying positive and negative chemotaxis, respectively

In figures 4 and 5 the plasmodium is advancing toward the test substance (oatmeal) located outside the microscopic field at the position indicated by the white dot. After the latter photograph was taken the attracting substance, oatmeal, was removed, and a repelling substance, a silicate (Lloyd's reagent), was substituted. Figures 6, 7 and 8 show the plasmodium as it reverses its form and direction of movement, being repelled by the silicate.

## SUMMARY

In order to supplement previous studies with leukocytes, the differences between positive and negative chemotaxis were investigated in a primitive free-living organism, *Physarum polycephalum*, a myxocyte. This organism, because of its large size, robustness and ease of handling, is better adapted to certain experiments than are leukocytes.

In gross preparations (visible to the naked eye) the substances tested included sugars, acids and alkalis. To dextrose chemotaxis was positive, to saccharose it was indifferent. Both hydrogen and hydroxyl ions in adequate concentrations induced negative chemotaxis, i. e., repelled the organism. Thus the positive or negative character of the reaction seems to depend on the chemical nature of the substance rather than merely on the concentration.

In microscopic preparations a small bit of the organism comparable to a macrophage was caused to alter its response from positive to negative. There was no qualitative difference in the behavior of the organism, when reacting negatively, it presented a mirror image of itself reacting positively. When altering its response the organism does not turn around but "goes into reverse."

It cannot be stated that the chemotactic responses of the myxomycete and leukocyte are identical, but so similar are their mode of locomotion and chemotactic behavior that the information gained from the myxomycete should be helpful in further studies of negative chemotaxis in leukocytes.

# INDUCED PULMONARY TUMORS IN MICE

## I SUSCEPTIBILITY OF SEVEN STRAINS OF MICE TO THE ACTION OF INTRAVENOUSLY INJECTED 20-METHYLCHOLANTHRENE

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BETHESDA, MD

The primary pulmonary tumor, next to mammary carcinoma, is the most common type of neoplasm in mice. The various inbred strains of mice that have been established in laboratories throughout the world show marked differences in susceptibility to the occurrence of pulmonary tumors, and it has been asserted that the susceptibility is a genetic dominant characteristic.<sup>1</sup> The tumors can be induced by the introduction of carcinogenic hydrocarbons into the animals. The susceptibility to the induction of primary pulmonary tumors varies markedly in different strains of mice and is parallel to their susceptibility to the spontaneous occurrence of growths, i. e., the mice most susceptible to the spontaneous occurrence of tumors of the lungs are also most susceptible to the induction of pulmonary tumors with carcinogens.<sup>2</sup>

In 1938 Andervont<sup>3</sup> published a study on the comparative susceptibility of eight inbred strains of mice to the spontaneous occurrence, the induction and the transplantation of tumors. The susceptibility to induction of primary pulmonary tumors was determined from animals given 1, 2, 5, 6-dibenzanthracene subcutaneously.

This is a report of a similar investigation, comparing the susceptibility of seven inbred strains of mice to the induction of primary pulmonary tumors by intravenously injected 20-methylcholanthrene. The intravenous route of injection is more advantageous for this determination because the dose of the hydrocarbon coming in contact with the lungs is directly ascertainable and because tumors at the sites of subcutaneous injection are avoided. The study was undertaken to ascertain whether the use of a different carcinogenic hydrocarbon and a different

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1 Bittner, J. J. Pub. Health Rep. **53** 2197, 1938

2 Andervont, H. B. Pub. Health Rep. **54** 1512, 1939

3 Andervont, H. B. Pub. Health Rep. **53** 1647, 1938



route of administration would influence the results and to compare the histologic appearance of the pulmonary tumors elicited in the various strains of mice

#### EXPERIMENTAL PROCEDURE

The experiment was started in December 1938. Mice of strains C, I, Y, C<sub>3</sub>H and C57 black were born and raised in this laboratory, and mice of strains A and L (or strain M [leaden]) were obtained from the Roscoe B. Jackson Memorial Laboratory. The animals were kept under identical environmental conditions. With the exception of the C57 blacks, a strain in which only males were available, equal numbers of males and females were used.

When 2 to 3 months of age, 38 A, 33 C, 27 I, 24 Y, 26 C<sub>3</sub>H, 21 C57 black and 22 L strain mice were each given intravenously, in the lateral tail vein, an injection of 0.5 mg. of methylcholanthrene dispersed in 0.5 cc. of horse serum and cholesterol. The dispersion was prepared according to a technique described previously.<sup>4</sup>

Five or more animals of each strain were killed and examined six, thirteen, twenty, twenty-six and thirty-two weeks after the injection. The lungs were fixed in Tellyesniczky's fluid and were examined grossly for pulmonary nodules; nodules visible on the external surfaces of the lungs were counted. Single sections of the lungs were stained with hematoxylin and eosin.

#### RELATIVE SUSCEPTIBILITIES OF SEVEN STRAINS OF MICE

Five mice died of intercurrent infection in the course of the experiment, and data on these were not included in the study. At post-mortem examination, none of the mice used in this study was observed in the gross to have tumors other than pulmonary tumors. In about half of the C57 black mice, however, chronic pneumonia developed, with large white patches of inflammatory reaction in the lungs.

Table 1, presenting the results, shows that mice of strain A are much more susceptible to the induction of pulmonary tumors by intravenous injection of methylcholanthrene than any of the other six strains. In six weeks all of the animals showed multiple tumors of the lungs. As a matter of fact, 0.5 mg. of methylcholanthrene introduced intravenously induced an average of 5 pulmonary tumors in 80 per cent of strain A mice four weeks after injection.<sup>5</sup> The number of tumors in each pair of lungs increased as the time after injection progresses. Thus, at six weeks each mouse had from 8 to 48 nodules, or an average

4 (a) Lorenz, E., and Andervont, H. B. *Am. J. Cancer* **26**: 783, 1936.

(b) Andervont, H. B., and Lorenz, E. *Pub. Health Rep.* **52**: 637, 1937. The dispersion was prepared by Dr. Lorenz. The hydrocarbon was a synthetic product with a melting point of 179.8-180.4 C. (corrected), and the final concentration was checked by absorption spectrum analysis.

5 Shimkin, M. B. *Arch. Path.*, this issue, p. 239.

TABLE 1—*Susceptibility of Seven Strains of Mice to a Single Intramuscular Injection of 0.5 Mg of Methylcholanthrene*

Strain of Mouse	Spontaneous Incidence of Tumors in Lungs of Mice over 1 Year Old, %	Susceptibility to Induction of Pulmonary Tumors by Subcutaneous Injection of Dibenzanthracene <sup>1</sup>	6 Weeks			13 Weeks			20 Weeks			26 Weeks			32 Weeks		
			Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice	Number of Mice Given Injection	Number Having Tumors of Lungs	Average Number of Tumors in Affected Mice
A	70-85	Very high	10	10	25	25	12	30	6	6	47	6	6	13	11	11	10
C	20-30	High	5	0	0	5	0	0	6	6	1	6	6	13	11	11	10
I	10-20	Medium				5	0	0	6	2	1	16	12	3			
X	10-20	Medium	3	0	0	3	1	1	7	2	1	10	6	1			
C <sub>3</sub> H	5-10	Medium	3	0	0	3	0	0	7	0	0	13	6	2			
C57 black	0-1	Low				7	0	0	7	0	0	6	1	1			
L		Low				7	0	0	7	0	0	7	0	0	5	1	2

of 25 nodules per animal, and at twenty weeks the average number of pulmonary tumors per strain A mouse was 47. No pulmonary tumors were seen in the other strains at six weeks, nor at thirteen weeks except for a single pulmonary nodule in a strain Y mouse.

Strain C is next in order of susceptibility to development of tumors of the lung, which appeared in animals killed twenty weeks after injection of the hydrocarbon. An increase in the number of pulmonary tumors per animal as the time after injection increased was also evident in this strain.

Strains I and Y are of medium susceptibility, the I mice are slightly more susceptible than the Y animals. Tumors began to occur in mice killed at twenty weeks. Six weeks later about 60 per cent of the animals showed nodules, and the affected mice had an average of about 3 nodules per pair of lungs. The  $C_3H$  strain is less susceptible than the I or Y strains, tumors were observed in half of the animals twenty-six weeks after injection.

Strains C57 black and L are comparatively resistant to the induction of tumors of the lung. One tumor appeared in a C57 black mouse in twenty-six weeks, and two tumors in a mouse of the L strain in thirty-two weeks.

Iball<sup>6</sup> proposed that the relative potency of carcinogenic compounds can be expressed by the index  $\frac{P}{T} \times 100$ , in which P is the percentage of animals in which tumors develop and T the average latent period in days. A similar index can be devised for the relative susceptibility of various strains of mice to pulmonary tumors  $\frac{P \times N}{T} \times 100$ , in which P is the percentage of animals in which tumors of the lungs develop, N the average number of pulmonary tumors per tumor-bearing animal, and T the *minimal* latent period for the appearance of tumors of the lungs in days. According to this index, the relative susceptibilities of the seven strains of mice to 0.5 mg of methylcholanthrene injected intravenously can be expressed as: strain A, 1,400, strain C, 300, strains I and Y, 100, strain  $C_3H$ , 50, and strains C57 black and L, 10.

#### PATHOLOGIC OBSERVATIONS

Primary pulmonary tumors in mice have been described on numerous occasions, and Tyzzer's report<sup>7</sup> is still unexcelled. Recently, Grady and Stewart,<sup>8</sup> of this laboratory, studied the histogenesis of the induced pulmonary tumors in strain A mice, the evidence is that at least the great

6 Iball, J. *Am J Cancer* **35** 188, 1939.

7 Tyzzer, E. E. *J M Research* **21** 479, 1909.

8 Grady, H. G., and Stewart, H. L. *Am J Path*, to be published.

majority of these neoplasms arise from the cells of the alveoli and not from the bronchi. They are adenomatous tumors extremely uniform in appearance. The nodules situated usually just below the pleura and connected with the thickened pleura consist of moderately large round or cuboid cells with definite boundaries and large round or oval nuclei. The growth is fairly solid and in many areas the cells are arranged around a central cavity suggesting alveolar arrangement. Blood vessels are few and mitoses are rare. The tumors are not encapsulated.

The induced tumors after appearing rather suddenly grow slowly, so that in six months the nodules may progress from pinpoint size to a diameter of a few millimeters. Many of the older tumors have the same appearance as the younger ones, but some show morphologic changes: the cells become larger, the tumor is more glandular and papillary, and occasional cystic areas are encountered. The larger tumors may lie in contact with bronchioles, but actual invasion of these structures by the tumor is not common.

It is believed that practically all these tumors are identical and they are therefore referred to simply as "adenomatous tumors."

Slye, Holmes and Wells<sup>9</sup> in a study of 160 spontaneous pulmonary tumors in mice, classified 20 as "unquestionably carcinomas," 43 as showing "a reasonably sure malignant tendency," 41 as of "doubtful malignancy" and 56 as "benign." It is possible that the neoplasms originate as nonmalignant tumors and then change more or less rapidly into the malignant type.<sup>10</sup> That all these tumors either originally or eventually are malignant is suggested by the following: (1) They are not encapsulated and they invade the lung tissue, (2) there is progressive growth, although actual death of the animal cannot be attributed to them, (3) they change in morphologic nature with age, (4) they are transplantable to mice of the same strain, and in the subcutaneous tissues often assume a sarcomatous appearance,<sup>11</sup> and (5) metastases outside the lungs, although rare, have been demonstrated.<sup>12</sup>

In this study, only single sections of the lungs were taken, a final decision concerning many points has to await observations on serial sections. One hundred and twenty-six individual induced primary pulmonary tumors, occurring in 78 mice, were gathered: some from the pathologic collection at this laboratory, so that tumors induced in various

9 Slye, M., Holmes, H. F., and Wells, H. G. *J. M. Research* **30** 417, 1914.

10 Campbell, J. A. *Brit. J. Exper. Path.* **18** 215, 1937.

11 Andervont, H. B. (a) *Pub. Health Rep.* **52** 347, 1937. (b) **54** 1519, 1939.

12 Slye and others.<sup>9</sup> Campbell.<sup>10</sup>

ways and by dibenzanthracene as well as by methylcholanthrene were included. Twelve spontaneous single tumors were also considered (table 2).

All of the pulmonary tumors, with the questionable exception of 2 tumors in C<sub>3</sub>H female mice in which the possibility of metastases from tumor of the breast could not be excluded, were remarkably uniform in appearance (figs 1 and 2). A few older tumors, which had been present over seven months, looked slightly different, of these, 4 had a cystadenomatous structure, and 2 contained spindle cells as well as the adenomatous cells. Of the 138 tumors, 111 were directly subpleural, and thickening of the pleura was noted with 84. Nineteen of the tumors, 12 of which were seven months old or older, were noted to have mitoses, more than one mitotic figure, up to eight, were present in only 5 tumors,

TABLE 2—*Pulmonary Tumors in Mice Examined Histologically*

Strain	Mice	Number Induced Tumors of Lungs	Number Spontaneous Tumors of Lungs	Number Subpleural Tumors	Number Tumors with Thickened Pleura	Number Tumors Showing Mitoses
A	41	67	6	57	43	5
C	16	30		27	20	8
I	10	8	2	7	3	1
Y	9	7	3	8	6	2
C <sup>3</sup> H	8	8	1	7	7	3
C57	5	5		4	4	0
L	1	1		1	1	0
Total	90	126	12	111	84	19

all from mice killed at least six months after the introduction of the carcinogen.

There was no difference of appearance (except that due to age) between (1) spontaneous and induced tumors, (2) tumors of the six strains of inbred mice, (3) tumors produced by methylcholanthrene and tumors produced by dibenzanthracene, (4) tumors produced by different routes of injection (subcutaneous, intravenous or intratracheal<sup>13</sup>), (5) tumors induced with the carcinogen in different physical states (dissolved in lard, dispersed in horse serum, as cholesterol pellet, or adsorbed on charcoal). Andervont<sup>14</sup> reported that pulmonary tumors produced in strain A mice with 2-amino-5-azotoluene or 3,4,5,6-dibenzcarbazole are also similar macroscopically and microscopically to tumors induced by the hydrocarbons.

13 Shimkin, M. B. Am J Cancer **36** 538, 1939.

14 Andervont, H. B. Pub Health Rep **54** 1529, 1939.

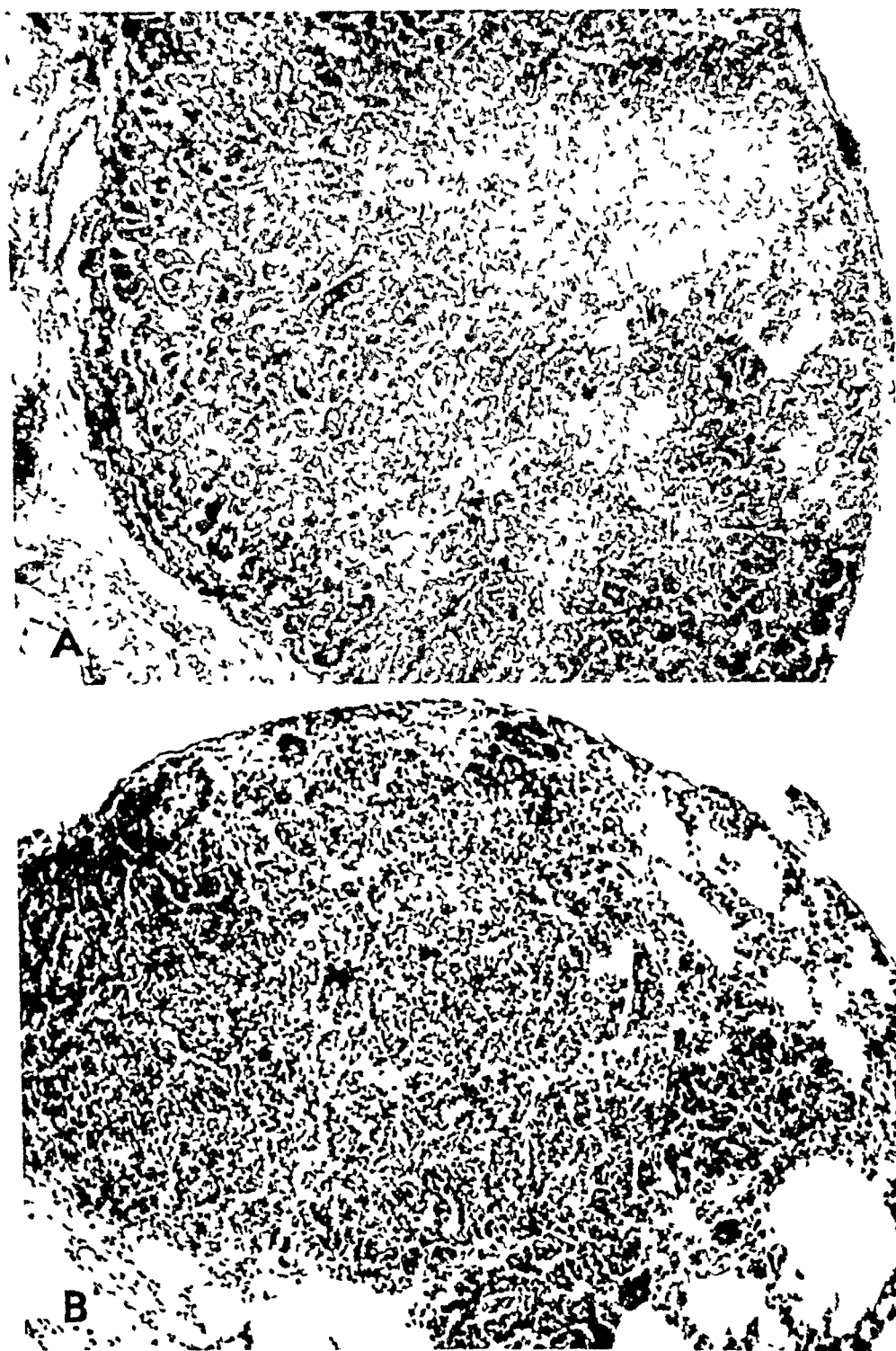


Fig 1—*A*, spontaneous pulmonary tumor in a male of strain C<sub>57</sub>H 15 months old, hematoxylin and eosin,  $\times 50$  *B*, induced pulmonary tumor in a male of strain C57 black twelve months after subcutaneous injection of 0.8 mg of 1,2,5,6-dibenzanthracene in lard, hematoxylin and eosin,  $\times 100$

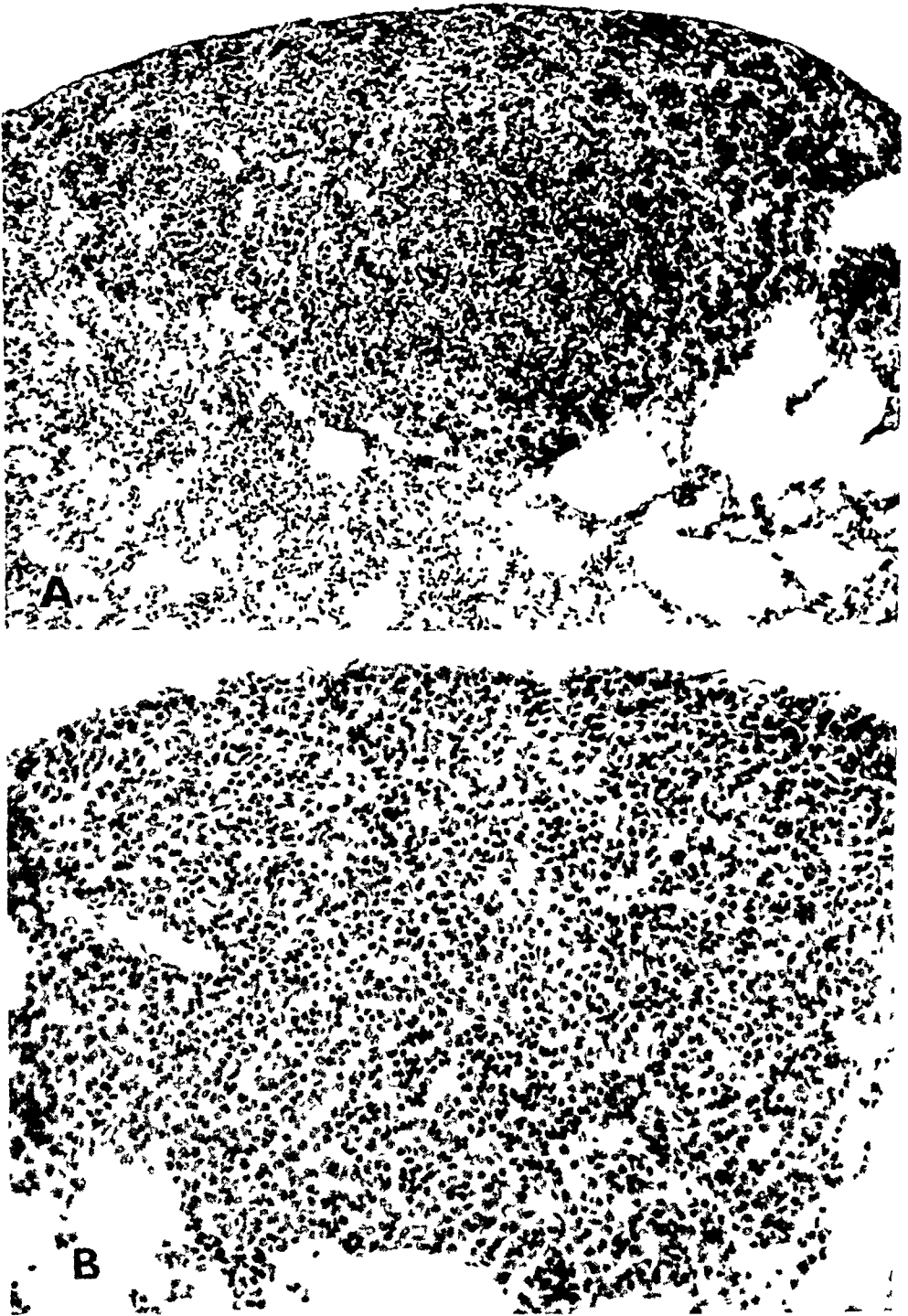


Fig 2—*A*, induced pulmonary tumor in a male of strain Y five months after subcutaneous injection of 2 mg of methylcholanthrene in laud, hematoxylin and eosin,  $\times 100$  *B*, induced pulmonary tumor in a female of strain A, three months after intravenous injection of 0.5 mg of methylcholanthrene in horse serum hematoxylin and eosin,  $\times 200$

## COMMENT

The relative susceptibilities of seven strains of mice to induction of primary pulmonary tumors by intravenously injected methylcholanthrene agree with the results obtained by Andervont<sup>15</sup> with subcutaneously injected 1,2,5,6-dibenzanthracene inlard. He determined that strain A mice were highly susceptible to the induction of pulmonary tumors by this agent and that the C strain was next in susceptibility, strains I, C<sub>H</sub> and Y were designated as of medium susceptibility, and strains C57 black, L and D as resistant. Tumors of the lungs were induced in all strains, however, including the three resistant ones.<sup>16</sup>

The use of methylcholanthrene rather than 1,2,5,6-dibenzanthracene, and the use of the intravenous route of injection instead of the subcutaneous, therefore, did not influence the results.

It is also evident that the susceptibility of the strains to induction of pulmonary tumors is parallel to their susceptibility to spontaneous development of pulmonary tumors. Thus, over 70 per cent of strain A mice over a year old have tumors of the lungs<sup>1</sup>, even at six months the incidence is about 10 per cent.<sup>16</sup> The great majority of the spontaneous tumors in strain A mice as in the other strains, are single, in contrast with the multiple induced tumors.

In strain C mice over a year old the incidence of spontaneous tumors of the lungs is 20 to 30 per cent. Recent data obtained by Andervont<sup>16</sup> show that in 187 strain I mice of an average age of 15 months 30 pulmonary tumors were seen (16 per cent) and that of 110 strain Y mice of an average age of 13 months 11 (10 per cent) had pulmonary tumors. Of 165 C<sub>H</sub> mice about 14 months old 11 (7 per cent) had tumors of the lungs.<sup>17</sup> The reported incidence of spontaneous pulmonary tumors in strain C57 black over a year old is less than 1 per cent.<sup>18</sup> The incidence of pulmonary tumors in the L mice has not been recorded, but the strain is related to the C57 and is resistant to spontaneous tumors of the lungs.<sup>15</sup>

The parallelism between the susceptibility to spontaneous development of pulmonary tumors and that to induction of pulmonary tumors with carcinogenic hydrocarbons suggests that the hydrocarbons are accelerators of some process inherent in the animals. It is interesting that the estrogens have a similar action, male mice of strains in which the females are highly susceptible to spontaneous development of mammary cancer readily acquire mammary tumors on treatment with estrogens, while male

15 Andervont, H. B. Pub. Health Rep. **54** 1524, 1939.

16 Andervont, H. B. Unpublished data.

17 Andervont, H. B. Pub. Health Rep. **54** 1158, 1939.

18 Little, C. C., Murray, W. S., and Cloudman, A. M. Am. J. Cancer **36** 431, 1939.



mice of strains in which the females have a low incidence of this type of tumor are much more resistant to the agent <sup>19</sup> However, with sufficient carcinogen or estrogen and sufficiently long periods of time, pulmonary tumors can be elicited with the former and mammary tumors with the latter even in the resistant strains of animals <sup>20</sup> The difference in susceptibility of various strains of mice to induction of pulmonary tumors is apparently a matter of degree, and, as Andervont stated,<sup>2</sup> hereditary factors exert their influence by controlling the degree of susceptibility

#### SUMMARY

Seven strains of mice were tested for their susceptibility to induction of primary pulmonary tumors by the intravenous injection of 20-methylcholanthrene. Strain A is most susceptible, strain C is next in susceptibility, strains Y, I and C<sub>3</sub>H are of medium susceptibility, and strains C57 black and L are relatively resistant.

The relative susceptibility to induction of primary pulmonary tumors is parallel to the susceptibility to spontaneous development of pulmonary tumors, i. e., mice which show the greatest number of induced pulmonary tumors also show the greatest number of spontaneous tumors of the lungs.

The pulmonary tumors induced in mice are adenomatous and almost identical in appearance. There is no morphologic difference (1) between spontaneous and induced tumors, (2) between tumors induced in the seven strains of mice, (3) between tumors produced by methylcholanthrene and those produced by 1,2,5,6-dibenzanthracene, (4) between tumors induced by different routes of injection (subcutaneous, intravenous and intratracheal) or (5) between tumors induced by the carcinogen in different states (in lard, in horse serum, as cholesterol pellet or adsorbed on charcoal).

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19 Gardner, W. U. *Arch Path* **27** 138, 1939.

20 Twombly, G. H. *Proc Soc Exper Biol & Med* **40** 430, 1939.

# INDUCED PULMONARY TUMORS IN MICE

## REACTION OF LUNGS OF STRAIN A MICE TO CARCINOGENIC HYDROCARBONS

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The inbred strain of albino mice designated as strain A was established by Strong,<sup>1</sup> in 1921. The breeding females have a high incidence of mammary carcinoma and both males and females are very susceptible to the development of primary pulmonary tumors<sup>2</sup>; the occurrence of other types of neoplasm in the stock has also been recorded.<sup>3</sup>

The lungs of strain A mice are extremely susceptible to the induction of tumors with carcinogenic agents and are a desirable tissue for many phases of investigation of the action of cancer-provoking compounds. This paper describes the response of the lungs of strain A mice to three carcinogenic polynuclear aromatic hydrocarbons, with special reference to the effects of dose, of the route of injection, of the medium for the compounds and of the time after injection of the appearance of the induced primary pulmonary tumors. The studies are based on previous work done in this laboratory by Dr. H. B. Andervont<sup>4</sup> and were initiated in December 1938.

### EXPERIMENTAL PROCEDURE

The inbred strain A mice used in these investigations were obtained from the Roscoe B. Jackson Memorial Laboratory, of Bar Harbor, Maine. They were maintained on Purina dog chow,<sup>5</sup> with an unlimited supply of water, and under

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From the National Cancer Institute, United States Public Health Service

1 Strong, L. C. *J. Hered.* **27** 21, 1936

2 Bittner, J. J. (a) *Am. J. Cancer* **27** 519, 1936, (b) *Pub. Health Rep.* **53** 2197, 1938, **54** 380, 1939

3 Cloudman, A. M., Bittner, J. J., and Little, C. C., in *Some Fundamental Aspects of the Cancer Problem*, Symposium Sponsored by the Section on Medical Sciences of the American Association for the Advancement of Science, New York, The Science Press, 1937, p. 37

4 Andervont, H. B. (a) *Pub. Health Rep.* **52** 212, (b) 304, (c) 347 and (d) 1584, 1937, (e) **53** 229, 1938, (f) **54** 1512, (g) 1519, (h) 1524 and (i) 1529, 1939

5 According to the manufacturer, the chow contains the following ingredients: protein, 20 per cent; fat, 3 per cent; carbohydrate, 56 per cent; ash, 6 per cent; and water, 15 per cent, with vitamins A and G added.

identical environmental conditions. They were from 2 to 3 months of age at the start of the experiments, and the experimental groups usually included equal numbers of males and females, which were kept separate.

The carcinogens employed were 20-methylcholanthrene, 1,2,5,6-dibenzanthracene and 3,4-benzpyrene.<sup>6</sup> The compounds were purified by Dr. J. L. Hartwell, and the melting points were 179.8-180.4 C, 266.0-266.8 C and 178.6-179.0 C (corrected) respectively. The dispersions of methylcholanthrene and dibenzanthracene in horse serum and cholesterol were prepared by Dr. Egon Lorenz according to a technique described previously,<sup>7</sup> and the final concentrations were checked by absorption spectrum analysis.

At designated periods after the injection of the carcinogens the animals were killed by cervical dislocation and autopsies made grossly. The lungs were examined for the presence and the number of white nodules and were fixed in Tellvesniczky's fluid. After fixation the nodules in the lungs, especially when small, became more evident, those in each pair of lungs were counted and the number recorded. The tissue was sectioned and stained with hematoxylin and eosin for histologic verification.

### RESULTS

Since the great majority of pulmonary tumors in mice is found directly underneath the pleura,<sup>8</sup> it was possible to count the tumors on the external surfaces of the lungs with gratifying accuracy, especially after fixation. Histologic sections attested that the gross appearance of the pulmonary tumors was characteristic: they were pearly, shiny, round areas, slightly raised and sharply distinct from the surrounding lung tissue (fig. 1). Occasional errors in the gross diagnosis were encountered in mice with chronic pneumonic infiltration of the lungs or with large lymphatic patches; in most of these, however, differentiation was easy, as the inflammatory or lymphomatous patches appeared as extensive, slightly nodular or smooth irregular grayish areas with ill defined boundaries.

The induced pulmonary tumors in strain A mice (and in other inbred strains as well) are morphologically identical with the pulmonary tumors of spontaneous origin except for their frequency and multiplicity.<sup>9</sup> They arise from the alveolar lining of the lung<sup>9</sup> and occur at the same site, just beneath the pleura. They are of the same macroscopic and microscopic appearance: adenomatous tumors which with advancing age often assume a papillary structure. Recognizable grossly when they are of pinpoint size, the tumors grow rapidly to a few millimeters in diameter, and then remain fairly stationary (figs

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6 Throughout the paper the compounds are referred to as methylcholanthrene, dibenzanthracene and benzpyrene.

7 (a) Lorenz, E., and Andervont, H. B. *Am. J. Cancer* **26**: 783, 1936. (b) Andervont, H. B., and Lorenz, E. *Pub. Health Rep.* **52**: 637, 1937.

8 Shimkin, M. B. *Arch. Path.*, this issue, p. 229.

9 Grady, H. G., and Stewart, H. L. *Am. J. Path.*, to be published.

1 and 2), death of the animal is not attributable to their presence. Inflammatory reaction is significantly absent in and around the tumors.<sup>10</sup> The site and the morphologic character of the induced pulmonary tumors are not altered by variations in the carcinogen, the medium of the carcinogen or the route of injection.<sup>5</sup>

Both the spontaneous and the induced pulmonary tumors are transplantable in the subcutaneous tissue of mice of the same strain where they often undergo a remarkable change in appearance and become spindle cell tumors.<sup>11, 12</sup>

That these pulmonary tumors are originally or eventually malignant is suggested by their invasion of the lung and lack of encapsulation.

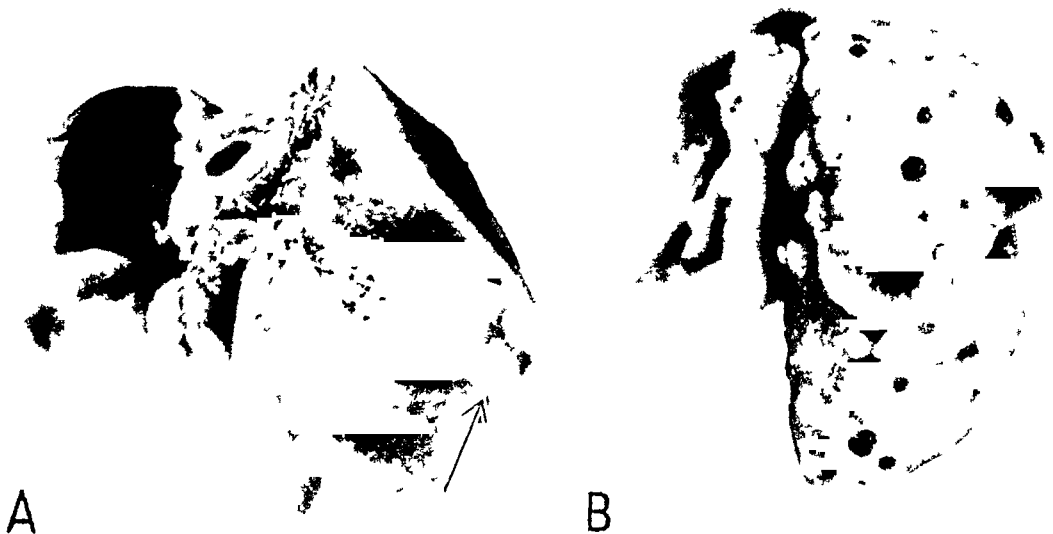


Fig. 1—*A*, spontaneous pulmonary tumor in strain A male mouse 8 months old  $\times 35$ . *B*, induced multiple pulmonary tumors in strain A male mouse thirteen weeks after an intravenous injection of 15 mg of methylcholanthrene dispersed in 15 cc of horse serum and cholesterol,  $\times 35$ .

their progressive growth and change in appearance, their transplantability and the demonstration of occasional metastases outside the lung.<sup>10</sup>

*Experiment 1 Incidence of Spontaneous Lung Tumors*—Knowledge of the incidence of spontaneous pulmonary tumors in strain A mice is an obvious essential for the interpretation of results with carcinogenic agents. Bittner<sup>2</sup> reported the incidence of pulmonary tumors in strain A mice of advanced age: at an average age of 148 months, 71.6 per cent have pulmonary tumors, and the incidence rises to 89.2 per cent at an average age of 195 months. This information, compiled according to the average age rather than month by month, does not meet the

<sup>10</sup> (a) Slye, M., Holmes, H. F., and Wells, H. G. *J. M. Research* **30**: 417, 1914. (b) Campbell, J. A. *Brit. J. Exper. Path.* **15**: 287, 1934; **18**: 215, 1937.

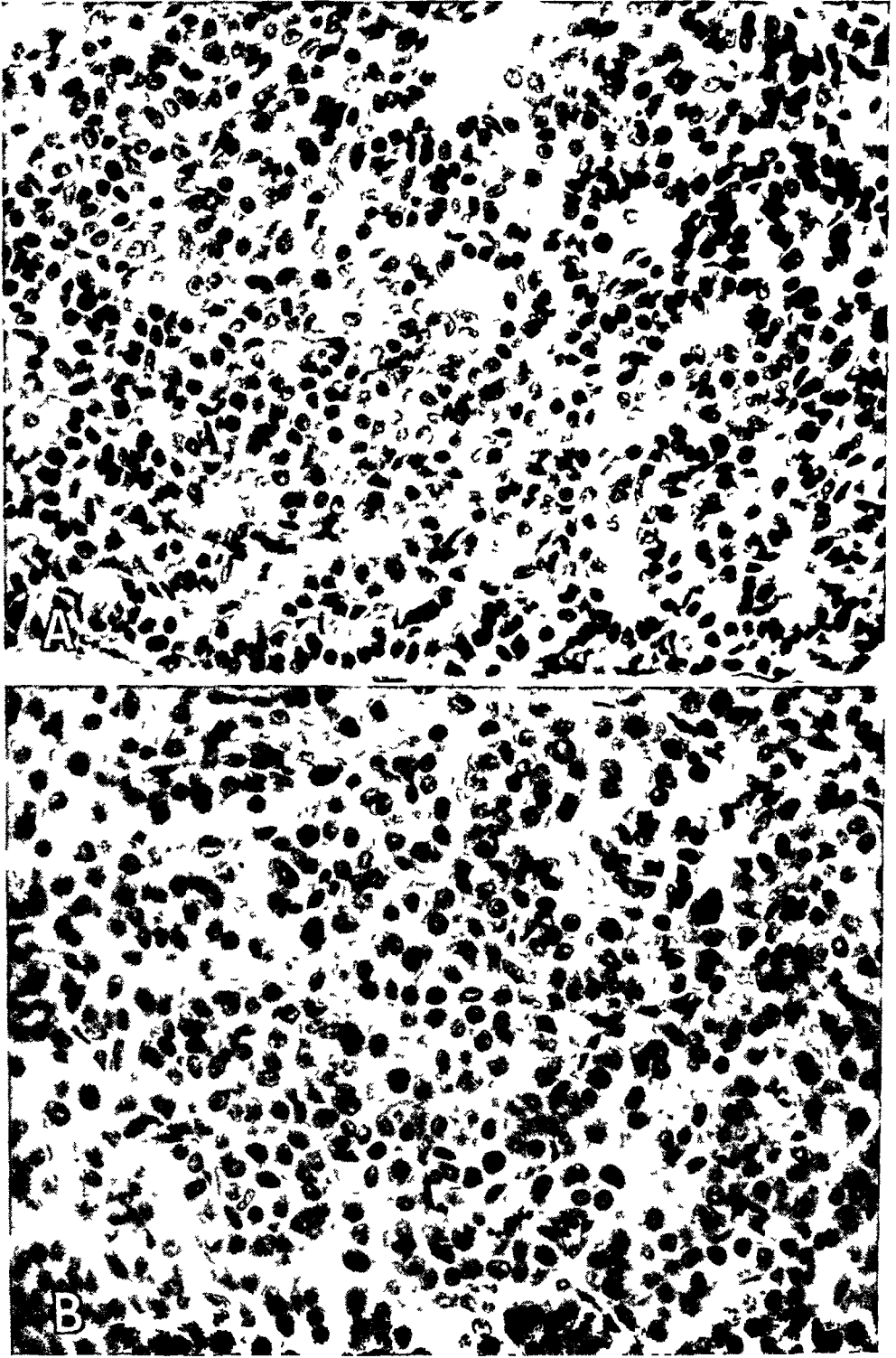


Fig 2—*A*, spontaneous pulmonary tumor in strain A male mouse 10 months old, hematoxylin and eosin,  $\times 200$  *B*, induced pulmonary tumor in strain A female mouse six weeks after intravenous injection of 0.5 mg of methylcholanthrene dispersed in 0.5 cc of horse serum and cholesterol, hematoxylin and eosin,  $\times 400$

requirement of experiments with carcinogens, which often are terminated long before the animals reach such ages.

The data on the incidence of spontaneous pulmonary tumors in strain A mice under 1 year of age were therefore gathered and supplemented by those from additional autopsies. The mice included both males and females, as sex does not influence the incidence of these tumors.<sup>1b</sup>

Table 1 shows that pulmonary tumors appear in strain A mice as young as 3 months and that the incidence rises gradually toward the figures given by Bittner. At 8 or 9 months of age, when the longest experiments described here were terminated, the incidence is 17 to 35 per cent.

Of special interest is the fact that few of the spontaneous pulmonary tumors in strain A mice are multiple. Thus up to 10 months of age only 1 animal with more than 1 pulmonary tumor was found in a total of 43 mice bearing tumors of the lungs. The incidence of multiple

TABLE 1—*Experiment 1 Incidence of Spontaneous Pulmonary Tumors in Strain A Mice Under 1 Year Old*

Age, Mo	Mice	Number with Tumors of the Lungs	Percentage with Tumors of the Lungs	Number with Multiple Tumors of the Lungs
3	40	1	2.5	0
4	25	1	4.0	0
5	20	2	6.8	0
6	43	4	9.3	0
7	75	11	14.7	0
8	52	9	17.5	1
9	41	15	31.5	0
10	45	21	42.2	0

tumors increases with advance in age, of 31 pulmonary tumors seen in mice over a year old (average age 15 months), 7 were multiple. Usually these mice have 2 tumors per pair of lungs, but up to 10 tumors in an animal 16 months of age have been observed. In judging whether pulmonary tumors in mice are induced by any given agent or are of spontaneous origin, the number of pulmonary tumors per animal as well as the incidence of such tumors in a group of animals must be taken into consideration.

*Experiment 2 Intravenous Injection of Methylcholanthrene*—Strain A mice 2 months of age, with an equal number of males and females in each group, were given intravenously, in a lateral tail vein methylcholanthrene dispersed in horse serum and cholesterol to equal 1 mg of the hydrocarbon per cubic centimeter of the serum, as follows:

(a) Forty animals received an injection of 0.5 cc, the amount tolerated without mortality. The dose was repeated in seven hours and again in sixteen hours, so that within twenty-four hours the mice received 1.5 mg of methylcholanthrene in 1.5 cc of horse serum. Eight mice died during the second and third injections.

(b) Sixty-four mice received a single injection of 0.5 mg of methylcholanthrene in 0.5 cc of horse serum. Two animals died of intercurrent infection before the termination of the experiment. The results in this group have been reported in part elsewhere<sup>8</sup>

(c) Thirty-three mice were given 0.1 mg of methylcholanthrene dispersed in 0.1 cc of horse serum. This dose was found previously to cause pulmonary tumors in strain A mice within five months<sup>11</sup>

The animals were killed at three, four, five, six, thirteen and twenty weeks after the injection of the carcinogen, and the incidence of pulmonary tumors in the group and the number of tumors per pair of involved lungs determined.

The results are summarized in table 2 and are recapitulated in graphic form in figure 3. Pinpoint tumors began to appear in the lungs within four weeks after the intravenous injection of 1.5 or 0.5 mg of methylcholanthrene. The number of tumors and the size of the individual tumors increased with progress of time after the injection, so

TABLE 2—Experiment 2. Incidence of Pulmonary Tumors in Strain A Mice After Intravenous Injection of Methylcholanthrene

Time, Weeks	1.5 Mg			0.5 Mg			0.1 Mg		
	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs
3	6	1	1	9	1	1			
4	7	7	12	10	8	5			
5	10	10	22	10	10	14			
6	4	4	55	10	10	25	7	3	2
13	5	5	74	18	15	30	10	8	4
20				6	6	47	15	15	11

that in three months the mice that received the larger dose had an average of 74 separate pulmonary tumors per pair of lung (fig 1B).

It is to be noted that the average number of pulmonary tumors per animal, as well as the number of mice bearing tumors, is directly proportional to the dose of the hydrocarbon administered. Thus, at four weeks all mice given 1.5 mg had tumors of the lungs, whereas 2 of 10 mice receiving 0.5 mg were uninvolved. At six weeks all of the animals receiving either dose had multiple tumors of the lungs, but with 1.5 mg the average number per mouse was 55, compared with 25 for animals receiving 0.5 mg. At this time, only half of the mice which had been given 0.1 mg had pulmonary tumors, and the tumor-bearing animals had an average of 2 such tumors.

It has been proposed<sup>8</sup> that the relative susceptibilities of various inbred strains of mice to induction of pulmonary tumors by carcinogenic hydrocarbons can be expressed by the index  $\frac{P \times N}{T} \times 100$ , in which

11 Shimkin, M. B. Am J Cancer 36: 538, 1939

P is the percentage of animals in which pulmonary tumors develop, N the average number of such tumors per tumor-bearing animal, and T the minimal time in days in which the tumors appear

In this experiment the validity of the index can be determined by the values obtained with the three doses of methylcholanthrene. For 1.5 mg, the index is 4,200, for 0.5 mg, 1,400, and for 0.1 mg, 250. The values are almost exactly proportional to the dose: 4,200 1,400 250 1.5 0.5 0.1.

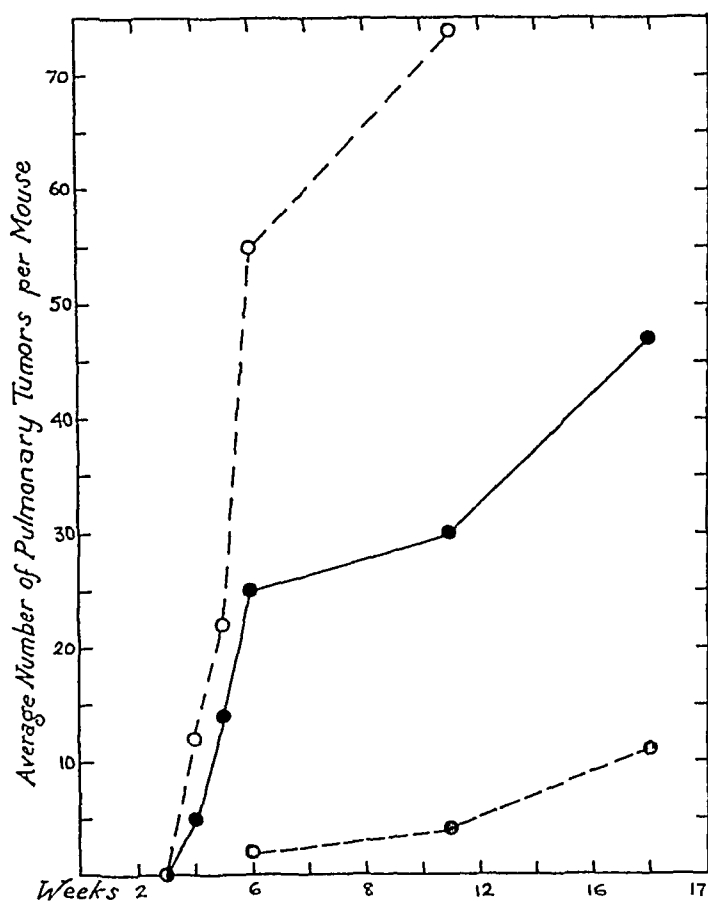


Fig 3—Response of the lungs of strain A mice to intravenously injected methylcholanthrene dispersed in horse serum. The dash line represents the response to 0.1 mg, the solid line, that to 0.5 mg and the dot-dash line that to 1.5 mg (from table 2).

*Experiment 3. Minimal Dose of Methylcholanthrene*—In order to ascertain the minimal intravenous dose of methylcholanthrene that would produce tumors of the lungs, groups of strain A mice 3 months old were given a single intravenous injection of the following dispersions of the compound in horse serum and cholesterol:

- (a) 0.1 mg of methylcholanthrene in 0.25 cc of horse serum (10 mice)
- (b) 0.05 mg of methylcholanthrene in 0.25 cc of horse serum (10 mice)



(c) 0.01 mg of methylcholanthrene in 0.25 cc of horse serum (20 mice)

(d) 0.001 mg of methylcholanthrene in 0.25 cc of horse serum (20 mice)

The animals given 0.1 mg of methylcholanthrene in 0.1 cc of horse serum (experiment 2) were included for comparison, and mice untreated or given injections of horse serum and cholesterol served as controls.

The mice were killed three to six months later, except for those given 0.001 mg and half of the controls, which were kept for another month, when they were 10 months old.

As presented in table 3, 0.05 mg of methylcholanthrene given intravenously induced tumors of the lungs in 90 per cent of the mice within three months.

TABLE 3—*Experiment 3. Minimal Dose of Methylcholanthrene Intravenously Required to Produce Tumors of the Lungs in Strain A Mice*

Dose of Methylcholanthrene, Mg	Vehicle	Vol., Cc	Mice Used	Time After Injection, Mo	Age of Mice at Death, Mo	Number Without Tumors of the Lungs	Mice Showing Given Number of Tumors per Mouse								Percentage of Mice with Tumors of the Lungs	Average Number of Tumors per Mouse
							1	2	3	5	6	10	11	20		
0.1	Horse serum	0.25	10	3	6	2		2	3	3					80	4
0.1	Horse serum	0.1	15	4.5	7.5	0		3	2	6		4	2		100	11
0.05	Horse serum	0.25	10	3	6	1	5	3			1				90	2
0.01	Horse serum	0.25	5	3	6	4	1								20	1
0.01	Horse serum	0.25	15	6	9	9	5	1							40	1
0.001	Horse serum	0.25	10	6	9	5	4	1							50	1
0.001	Horse serum	0.25	10	7	10	7	2	1							30	1
	Horse serum	0.25	10	6	9	9	1								10	1
	Horse serum	0.25	10	7	10	6	3	1							40	1
			10		9	9	1								10	1
			10		10	5	5								50	1

The interpretation of the results with smaller doses is difficult. A dose of 0.01 mg did not induce pulmonary tumors in three months, in six months the incidence was 40 per cent. At this age, 9 months, 10 per cent of the control animals had tumors of the lungs, it seems that the hydrocarbon accelerated their appearance. However, the 30 per cent incidence among the animals used to establish the occurrence of spontaneous pulmonary tumors (table 1) negates the finding.

From the data obtained, it can be concluded that 0.05 mg of methylcholanthrene given intravenously induced pulmonary tumors in strain A mice within three months, and that 0.01 mg perhaps accelerated their appearance. Concerning doses below 0.05 mg, it is felt that definite information cannot be derived from this experiment but could be elicited with greater numbers of experimental animals killed periodically in three to six months after injection.

*Experiment 4 Intravenous Injection of Dibenzanthracene*—It has been reported<sup>12</sup> that tumors of the lungs appear as quickly after the intravenous injection of dibenzanthracene as after that of methylcholanthrene. Since the carcinogenic index, which takes into consideration the multiplicity of induced pulmonary tumors as well as their incidence in the group and time of appearance, allows a more exact comparison of carcinogenicity and has been shown to be valid for three doses of methylcholanthrene (experiment 2), the following experiment was undertaken to evaluate the comparative cancer-provoking power of dibenzanthracene and methylcholanthrene. Percutaneous application and subcutaneous injection of the two compounds have established that methylcholanthrene is considerably more carcinogenic than dibenzanthracene in the induction of carcinomas and sarcomas.<sup>12</sup>

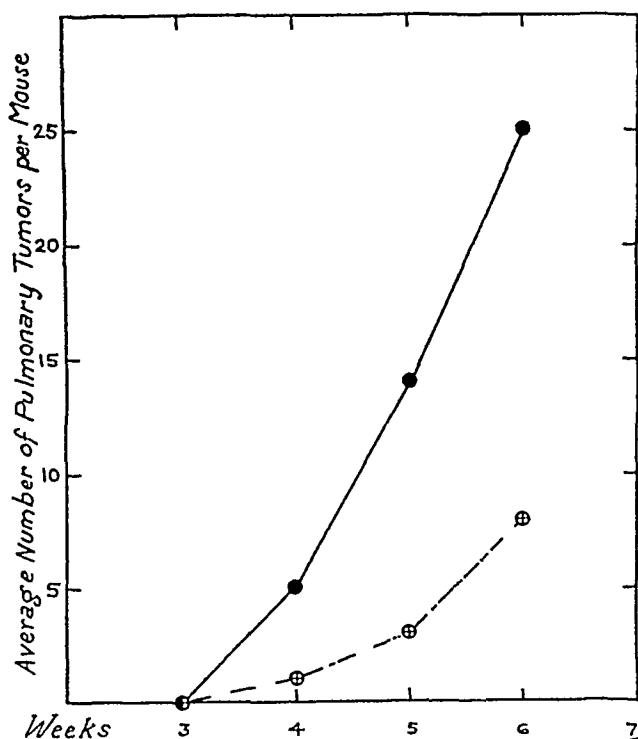


Fig 4—Response of the lungs of strain A mice to intravenously injected methylcholanthrene (solid line) and dibenzanthracene (broken line) dispersed in horse serum (from table 4)

Forty-one strain A mice  $2\frac{1}{2}$  months of age were given intravenously 0.5 mg of dibenzanthracene dispersed in 0.5 cc of horse serum and cholesterol. Ten animals were killed three, four, five and six weeks after injection and the lungs examined for the presence and the number of pulmonary tumors.

The results are given in table 4 and are compared with those obtained with the equivalent dose of methylcholanthrene, a graphic recapitulation is presented in figure 4. Tumors of the lungs appeared at approximately the same time as with methylcholanthrene, i. e., in four weeks. The incidence of pulmonary tumors in the group, as well as the average

<sup>12</sup> (a) Iball, J. *Am J Cancer* **36** 538, 1939. (b) Shimkin, M. B., and Andervont, H. B. *Pub Health Rep*, to be published.

number of tumors per animal, however, was significantly lower with dibenzanthracene. Thus, at four weeks 30 per cent had an average of 1 tumor per animal, as compared with 80 per cent with an average of 5 tumors with methylcholanthrene. At six weeks all animals that had received an injection of either hydrocarbon had tumors of the lungs, but the average number of tumors was significantly lower with dibenzanthracene.

The experiment indicates that dibenzanthracene is less carcinogenic than methylcholanthrene in inducing pulmonary tumors as well as in

TABLE 4—*Experiment 4 Incidence of Pulmonary Tumors in Strain A Mice After Intravenous Injection of 0.5 Gm of Methylcholanthrene or 0.5 Mg of Dibenzanthracene*

Time, Weeks	Methylcholanthrene, 0.5 Mg			Dibenzanthracene, 0.5 Mg		
	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs	Mice	Number with Tumors of the Lungs	Average Number of Tumors of the Lungs
3	9	1	1	10	0	0
4	10	8	5	10	3	1
5	10	10	14	11	10	3
6	10	10	25	10	10	8

TABLE 5—*Experiment 5 Incidence of Pulmonary Tumors in Strain A Mice Given Subcutaneously 0.5 Mg of Methylcholanthrene or 0.5 Mg of Dibenzanthracene Dispersed in 0.5 cc of Horse Serum and Cholesterol*

Time, Weeks	Methylcholanthrene			Dibenzanthracene		
	Mice	Number with Tumors of the Lungs	Average Number of Pulmonary Tumors	Mice	Number with Tumors of the Lungs	Average Number of Pulmonary Tumors
3	10	0	0	10	0	0
4	10	2	1.0	7	0	0
5	10	5	1.6	6	2	1.0
6	10	7	2.2	7	5	2.0

inducing cutaneous carcinomas or subcutaneous sarcomas. It is suggested that the time of appearance, the incidence and the average number of pulmonary tumors per mouse in strain A mice after the intravenous introduction of a carcinogen (i. e., the carcinogenic index) can be used in determining quantitatively the relative carcinogenic power of the carcinogen in comparison with other compounds. The rapidity with which data are obtained, as compared with subcutaneous or percutaneous testing, is an additional advantage.

*Experiment 5 Subcutaneous Injection of Methylcholanthrene or Dibenzanthracene Dispersion*—In order to ascertain the influence of the route of injection on the formation of pulmonary tumors, 40 strain A mice were given subcutaneously in the right axilla 0.5 mg of methylcholanthrene dispersed in 0.5 cc of horse serum

and cholesterol, and 30 strain A mice, the same amount of dibenzanthracene dispersion. The animals were killed at three, four, five and six weeks after the injection of the carcinogens and the lungs examined for the presence and the number of pulmonary nodules.

The results are presented in table 5. Pinpoint-sized tumors appeared in the lungs within four weeks after the injection of methylcholanthrene, and a week later with dibenzanthracene. The number of animals with pulmonary tumors and the average number of tumors per animal rose with progress of time after injection.

Although the slightly earlier appearance and the slightly greater average number of pulmonary tumors induced by methylcholanthrene

TABLE 6—Experiment 6. Incidence of Pulmonary Tumors in Strain A Mice Three Months After Subcutaneous Injection of Three Carcinogenic Hydrocarbons Dissolved in 0.25 cc of Lard

Hydrocarbon	Dose, Mg	Mice Given Injection	Number Without Tumors of the Lungs	Mice with Given Number of Tumors per Mouse								Percentage of Mice with Tumors of the Lungs	Average Number of Tumors per Mouse	Carcinogenic Index
				1	2	3	5	6	10	11	20			
Methylcholanthrene	0.25	10	3	3	2	2						70	1.8	125
Methylcholanthrene	0.5	10	2	2	2	4						80	2.5	200
Methylcholanthrene	1.0	11	1	1	3	4				2		91	4.3	390
Benzpyrene	0.25	10	6	3		1						40	1.5	60
Benzpyrene	0.5	9	6	3								33	1.0	35
Benzpyrene	1.0	10	5	2	2	1						50	2.2	110
Dibenzanthracene	0.25	10	3	4	2	1						70	1.8	125
Dibenzanthracene	0.5	10	0	3	1	5		1				100	2.6	280
Dibenzanthracene	1.0	10	0	1	1	5		2		1		100	4.4	440

suggest the greater carcinogenicity of the agent as compared with dibenzanthracene, the difference that is so evident with the intravenous route of administration is obscured. It is apparent that the subcutaneous route of injection is not satisfactory for the more exact determination of relative carcinogenicities. By the intravenous route the compounds come in immediate and direct contact with the pulmonary tissues, when the injections are made subcutaneously, the production of tumors of the lungs is modified not only by the relative carcinogenicity of the compounds but probably primarily by the ability of the agents to dissolve and to reach the lungs. Thus, subcutaneously injected chemicals which are slowly absorbed from the site of injection and which are rapidly destroyed or eliminated would have little opportunity to induce pulmonary tumors, whereas with intravenous injection these factors would be minimized.

*Experiment 6. Subcutaneous Injection of Methylcholanthrene, Dibenzanthracene or Benzpyrene in Lard*—Andervont<sup>4d</sup> reported that lard solutions of

dibenzanthracene are not as efficacious as serum dispersions in producing pulmonary tumors in mice, probably because the carcinogen is maintained more firmly at the site of injection. The following experiment was made to determine the influence of lard solution as compared with that of serum dispersion, as shown in experiment 5, and to study the effect of dosage on the incidence of tumors in the group and the average number of pulmonary tumors per mouse.

Groups of 10 strain A mice 2 months of age were given subcutaneously in the right axilla 0.25, 0.5 or 1.0 mg of methylcholanthrene, dibenzanthracene or benzpyrene dissolved in 0.25 cc of best grade lard filtered at 37 C. Three months later all the animals were killed and the lungs examined for tumors.

The results are summarized in table 6. Tumors of the lungs were induced in all groups of mice within three months. In contrast with the observations with the intravenous route of administration (experiments 2 and 4) and possibly with the subcutaneous injection of the horse serum dispersion (experiment 5), dibenzanthracene in lard injected subcutaneously produced pulmonary tumors in a greater percentage of mice as well as a greater number of pulmonary tumors per animal, than methylcholanthrene. Benzpyrene was definitely less carcinogenic in this vehicle and with this route of injection, as far as tumors of the lungs were concerned, than dibenzanthracene or methylcholanthrene.

The observations are of interest because benzpyrene is more carcinogenic than dibenzanthracene in producing subcutaneous sarcoma or carcinoma of the skin in mice, although less so than methylcholanthrene.<sup>12</sup> As a matter of fact, at the termination of the experiment, 9 of 11 mice receiving 1.0 mg of methylcholanthrene and 4 of 10 mice receiving 0.5 mg of methylcholanthrene had subcutaneous tumors at the sites of injection, whereas no subcutaneous sarcoma was encountered in the other groups.

It is apparent that with subcutaneously injected lard solutions of the three hydrocarbons there is no parallelism between the local carcinogenic power and the ability to produce tumors in the lungs. The reasons for the phenomenon are obscure, but are likely in part dependent on the physical state of the hydrocarbon, i. e., on the ability of the agent or its derivatives to be transferred from the locus of injection to the pulmonary tissue.

With all three compounds there is an accurate correlation between the dose of the hydrocarbon and the incidence and average number of induced pulmonary tumors. Thus, with 1.0 mg of dibenzanthracene all animals had an average of 4.4 tumors, with 0.5 mg, the mice had an average of 2.8 tumors and with 0.25 mg, 70 per cent of the mice had an average of 1.8 tumors. The carcinogenic index in this instance, with the time being constant, is derived by multiplying the percentage of animals in which tumors develop by the average number of tumors per tumor-bearing animal, the agreement between the dose and the index values is evident.

*Experiment 7 Time of Appearance of Pulmonary Tumors with Methylcholanthrene in Fatty Solvents*—In the course of investigations on the effect of various solvents on carcinogenesis with methylcholanthrene,<sup>13</sup> 53 strain A male mice each received a subcutaneous injection of 0.5 mg of methylcholanthrene dissolved in 0.25 cc of lard, tricaprylin, tricaproin, tricaprylin-trilaurin mixture or mouse fat. Each animal was killed as soon as an indubitable subcutaneous tumor appeared, and the lungs were examined for the presence and the number of pulmonary tumors.

The results, as far as tumors of the lungs were concerned, were identical with the different solvents and are summarized as one group in table 7. It is seen that tumors of the lungs began to appear in ten to twelve weeks in mice given 0.5 mg of methylcholanthrene in fatty solvents, and that the number of tumor-bearing animals as well as the average number of pulmonary tumors per mouse rose as the time after administration increased.

TABLE 7—*Experiment 7 Time of Appearance of Pulmonary Tumors in Strain A Mice Given Subcutaneously 0.5 Mg of Methylcholanthrene in Various Solvents*

Time, Weeks	Mice Killed	Number with Tumors of the Lungs	Percentage with Tumors of the Lungs	Average Num- ber Tumors per Mouse
8	6	0	}	0
10	10	0		
12	12	5		2
14	5	1	}	3
16	4	2		4
18	3	3	}	4
20	4	3		3
22-30	9	9	100	6

These data are comparable with the findings with subcutaneous injection of 0.5 mg of methylcholanthrene dispersed in horse serum (experiment 5). Pulmonary tumors appeared earlier and in greater number with the latter preparation, probably because of the greater diffusibility and ability to come in contact with the lung tissues.

A group of 10 strain A mice 2 months old, which are not included in the summary, were given subcutaneously 0.1 mg of methylcholanthrene in 0.25 cc of lard. They were killed six months later, 5 animals had single pulmonary tumors, and 2 had 2 pulmonary tumors each. The incidence of 70 per cent at eight months was sufficiently high to prove that this dose induced pulmonary tumors.

#### COMMENT

The induction of primary pulmonary tumors in animals is dependent on many factors, which can be divided into (1) the susceptibility of the animal and (2) the carcinogen.

13 Shimkin, M. B., and Andervont, H. B. Pub. Health Rep., to be published.

1 The susceptibility of the animal, a characteristic which is probably genetically determined, is of two types general susceptibility to the carcinogen and susceptibility of a specific organ. Thus, rabbits are extremely resistant to carcinogenic aromatic hydrocarbons,<sup>14</sup> while mice are extremely susceptible. Susceptibility of a specific organ can be illustrated by the failure to produce tumors of the lungs in rats by the intratracheal insufflation of methylcholanthrene,<sup>15</sup> while a similar procedure with strain A mice induced tumors of the lungs within five months,<sup>11</sup> both species are susceptible to subcutaneous sarcomagenesis with the agent.

The inbred strains of mice show a marked difference in their susceptibility to induced pulmonary tumors,<sup>16</sup> and this indicates the possible mode of action of the carcinogens on lung tissue. It has been demonstrated by means of subcutaneously injected dibenzanthracene<sup>16a</sup> and intravenously injected methylcholanthrene<sup>8</sup> that the strains of mice which are most susceptible to the development of spontaneous pulmonary tumors are also most susceptible to the induction of these tumors with the hydrocarbons, and vice versa. This suggests that the compounds are accelerators of some process inherent and genetically determined in the animals. It is true that tumors of the lungs can be induced in all strains of mice, including the resistant ones,<sup>4b</sup> but no strain of mouse which has been adequately studied at advanced ages is completely free of spontaneous occurrence of this type of neoplasm.<sup>17</sup>

In strain A mice the susceptibility to spontaneous development of tumors of the lungs has been shown to be a dominant genetic characteristic,<sup>2b</sup> this is also true of the induced tumors.<sup>4b, c</sup>

2 The factors concerning the carcinogen in the induction of pulmonary tumors can be summarized under the following headings

(a) The substances which have been found to produce pulmonary tumors in mice include tar, probably owing to its benzpyrene content, the three common polynuclear aromatic hydrocarbons, methylcholanthrene, dibenzanthracene and benzpyrene, 8,9-dimethyl-1,2-benzanthracene,<sup>18</sup> and 3,4,8,9-dibenzpyrene.<sup>18a</sup> Two compounds which are not hydrocarbons, 3,4,5,6-dibenzcarbazole and 2-amino-5-azotoluene, also have been reported<sup>4a</sup> to produce pulmonary tumors in strain A mice.

(b) The dose of the carcinogen influences the appearance of pulmonary tumors in mice. In this report it has been shown that the

14 (a) Burrows, H., and Boyland, E. *Am J Cancer* **32** 367, 1938. (b) Klinke, J. *Ztschr f Krebsforsch* **47** 341, 1938.

15 Valade, P. *Compt rend Acad d sc* **204** 1281, 1937.

16 (a) Andervont, H. B. *Pub Health Rep* **53** 1647, 1938. (b) Shimkin <sup>s</sup>

17 Little, C. C., Murray, W. S., and Cloudman, A. M. *Am J Cancer* **36**. 431, 1939.

18 Shear, M. J. *Am J Cancer* **28** 334, 1936.

18a Kleinenberg, H. E. *Arch Biol Sc (U S S R)* **51** 127, 1938.

number of animals showing tumors, the average number of tumors per animal and the time of appearance are directly proportional to the amount of hydrocarbon employed (fig 3 and table 6). Lettinga,<sup>19</sup> using a more resistant strain of mice than the A strain, found that five subcutaneous injections of 0.5 mg of dibenzanthracene produced multiple pulmonary tumors in the animals, that 0.05 mg injected five times raised the incidence of single pulmonary tumors and that doses below this were ineffective. The abrupt transition from a small to a large number of induced tumors suggests an overflow of the carcinogen from the actual site of injection as the cause of the genesis of tumors of the lungs.<sup>20</sup>

In strain A mice an intravenous injection of 0.05 mg of methylcholanthrene or a subcutaneous administration of 0.1 mg in lard induces pulmonary tumors. In this connection, dibenzanthracene injected subcutaneously in doses ample to induce tumors of the lungs has not been detected in the lungs of these animals.<sup>21</sup> The dose necessary to evoke pulmonary tumors in susceptible mice is therefore extremely small, or else the hydrocarbon undergoes alterations which destroy its absorption spectrum bands.

(c) The route by which the carcinogen is given modifies the induction of pulmonary tumors. Some of the methods of administration that have been used by various investigators are: repeated application of coal tar to the skin,<sup>22</sup> inhalation of dust containing tar,<sup>23</sup> subcutaneous injection of carcinogenic hydrocarbons,<sup>10a</sup> dibenzcarbazole or azotoluene,<sup>41</sup> intravenous injection of serum or charcoal dispersions of carcinogenic hydrocarbons,<sup>4d</sup> injection of the hydrocarbons into the peritoneal<sup>24</sup> or pleural cavity<sup>25</sup> or into the spleen,<sup>26</sup> insertion of a string impregnated with dibenzanthracene through the lung,<sup>4d</sup> intratracheal injection of the hydrocarbons dispersed in horse serum,<sup>11</sup> feeding large doses of dibenzanthracene emulsion in olive oil,<sup>27</sup> or injection of dibenzanthracene in olive oil into the stomach by means of a tube.<sup>28</sup> In the last method mentioned, there is direct aspiration of the carcinogen into the lungs.<sup>28</sup>

19 Lettinga, T. W. De carcinogene werking van kleine doses 1,2,5,6-dibenzanthraceen, Academisch, Proefschrift, Amsterdam, 1937.

20 Kennaway, E. L., and Kennaway, N. M. *Acta, Union internat. contra cancer* **2** 101, 1937.

21 Lorenz, E. Unpublished data.

22 Murphy, J. B., and Sturm, E. *J. Exper. Med.* **42** 693, 1925.

23 Campbell, J. A. *Brit. J. Exper. Path.* **15** 287, 1934.

24 Schabad, L. M. *Acta, Union internat. contra cancer* **3** 369, 1938.

25 Andervont, H. B., and Lorenz, E. *Pub. Health Rep.* **52** 1931, 1937.

26 Furth, J., and Furth, O. B. *Am. J. Cancer* **34** 169, 1938.

27 Lorenz, E., and Stewart, H. L. Unpublished data.

28 Magnus, H. A. *J. Path. & Bact.* **49** 21, 1939.



The most efficacious technic of inducing pulmonary tumors in mice, as far as the rapidity of appearance and multiplicity are concerned, is that of intravenous injection. Fewer tumors, arising later, are obtained with the subcutaneous and the intratracheal mode of administration.

(d) The medium in which the carcinogens are introduced into the animals also modifies their action. The production of pulmonary tumors is accelerated when a carcinogen is injected in a readily soluble form, as in dog or horse serum dispersions.<sup>4d</sup> Various solvents for the hydrocarbons, such as lard, glycerin, mouse fat, tricapion and tricaprylin, cause fewer and later-appearing pulmonary tumors than the serum preparations. Moreover, the medium exerts further effects, the explanation of which is obscure. The relative carcinogenicity of methylcholanthrene and dibenzanthracene are reversed when the compounds are injected as lard solutions rather than as dispersions in serum.

When the hydrocarbons are maintained firmly at the site of injection by the menstruum, as is true when they are in cholesterol pellets or adsorbed on charcoal,<sup>29</sup> few pulmonary tumors are produced. The role of the medium for the carcinogen is important evidence in support of the contention that pulmonary tumors in mice are induced through direct contact of the compound or of its active derivatives with the susceptible pulmonary tissue.

(e) The time of appearance of the induced pulmonary tumors depends on the susceptibility of the mouse, the carcinogen employed, the dose, the medium for the compound and the route of injection. With 1.5 mg of methylcholanthrene injected intravenously, 100 per cent of strain A mice have multiple tumors of the lungs in four weeks. With 0.5 mg of dibenzanthracene injected intravenously, pulmonary tumors begin to appear in four weeks also, but the percentage of tumor-bearing mice and the average number of tumors per mouse are reduced. Grady and Stewart<sup>9</sup> found a single tumor in a strain A mouse thirty-two days after subcutaneous injection of 0.8 mg of methylcholanthrene in lard, but at eleven weeks only 50 per cent of the animals had pulmonary tumors. In this investigation 0.5 mg of methylcholanthrene in lard failed to induce pulmonary tumors before the tenth week, and in twelve weeks the incidence was 40 per cent. Thus, the greatest number of tumors in groups of strain A mice or in any one mouse appear earliest with the largest doses of intravenously injected methylcholanthrene, the time of appearance and the incidence are slightly longer and less, respectively, for intravenously injected dibenzanthracene and for either hydrocarbon administered subcutaneously.

#### SUMMARY

Spontaneous pulmonary tumors, usually single, appear in strain A mice 3 months old, and the incidence rises sharply with advancing age.

29 Andervont<sup>4d</sup> Andervont and Lorenz<sup>25</sup>

Intravenously injected 20-methylcholanthrene induces multiple pulmonary tumors within four weeks, the number of mice showing tumors and the average number of tumors per pair of lungs is proportional to the dose of the hydrocarbon. Intravenously injected 1,2,5,6-dibenzanthracene induces pulmonary tumors as quickly as 20-methylcholanthrene, but the incidence in the group and the average number of tumors per mouse are significantly lower.

The definite difference in the power of 20-methylcholanthrene and 1,2,5,6-dibenzanthracene to produce tumors of the lungs is obliterated when the compounds are injected subcutaneously, although the incidence in the group and the number of pulmonary tumors per mouse induced is still proportional to the dose of the hydrocarbon administered. Pulmonary tumors appear later with larger solutions of the compounds than with the colloid suspensions injected subcutaneously.

Lungs of strain A mice are an admirable tissue for many phases of investigations on the action of cancer-provoking chemicals.

# PERICARDIAL MILK SPOTS

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Localized white opaque fibrous thickenings of the visceral pericardium (milk spots, tendinous patches, soldiers' spots, maculae tendineae, *Sehnenflecke*, etc ), most commonly seen on the anterior surface of the right ventricle, have been described and their origin disputed since at least 1806<sup>1</sup> Their genesis has been held to be either inflammatory, mechanical or both, and there have been about equal numbers of adherents of the three concepts There have been a few more or less successful experimental productions of these spots in animals Numerous descriptions of the gross and microscopic appearance of the spots in man have been published, but data concerning their frequency, age distribution, anatomic location and association with various diseases are practically nonexistent, and it is therefore the purpose of this paper to present such data

## MATERIAL

The observations in this paper are derived from a series of 494 autopsies performed by myself<sup>2</sup> after I had become interested in this subject Excluded from this number are 50 autopsies on infants under 1 year of age and 13 on older persons with obscuring lesions, such as diffuse pericarditis or pericardial adhesions Only definite localized epicardial thickenings of 1 cm or greater diameter (except for 6 between 7 mm and 1 cm ) were included, the irregular thickenings frequently seen over the coronary vessels were not included

## GROSS OBSERVATIONS

Of the 494 persons 1 year or more of age, 170 (34.4 per cent) showed one or more pericardial milk spots (table 1) Of the 439 persons 18 years or more of age, 165 (37.6 per cent) showed spots, leaving the remaining 5 cases (9.1 per cent) in the group of 55 persons between 1 and 18 years of age (table 2)

*Incidence by Age and Sex*—The distribution of milk spots by age groups is given in table 3 The incidence varies from 9.1 per cent in the

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From the National Institute of Health, United States Public Health Service

1 Tsunoda, T Frankfurt Ztschr f Path 3 220, 1909

2 The autopsies were performed at the University of Minnesota, and the microscopic sections were made there The compilation of the data was done in Washington

group from 1 to 18 years of age to 73.4 per cent in the group 80 or more years of age, the latter group is rather small, however, and throughout most of the life span, from 30 to 80 years, the incidence varies between 32 and 42 per cent. The groups in this age range are large enough to make the figures of value. If the production of milk spots were on a purely mechanical or an age basis, there should be a definite and marked increase of incidence with age, this series does not show such an increase.

In the literature the only figures for the incidence of milk spots are those of Tsunoda,<sup>1</sup> who stated that the incidence in the first decade was 8.5 per cent, in the second 10.0 per cent, in the third 23.0 per cent, in

TABLE 1—*Incidence of Pericardial Milk Spots in Age Groups*

	18 Years and Over		1 to 18 Years		All Ages	
	Subjects	Percentage of Age Group	Subjects	Percentage of Age Group	Subjects	Percentage of Age Group
With spots	165	37.6	5	9.1	170	34.4
Without spots	274	62.4	50	90.9	324	65.6
Total	439	100.0	55	100.0	494	100.0

TABLE 2—*Data on Subjects from One to Eighteen Years of Age*

Sex	Age, Years	Cause of Death	Size of Milk Spot	Location	Weight of Heart, Gm
M	2½	Hydronephrosis	3 patches, largest 1 cm	Various	70
M	5	Tumor of brain	8 mm	Left ventricle	75
F	7	Burns	8 mm	Left apex anterior	110
M	10	Lymphatic leukemia	7 mm	Left ventricle anterior	140
M	15	Tumor of brain	1 cm	Right ventricle anterior	180

the fourth 28.0 per cent, in the fifth 47.0 per cent, in the sixth 54.0 per cent and later than the sixth decade 65.0 per cent. Unfortunately, the number of cases on which this series was based was not given. Adam,<sup>3</sup> stated simply that they are to be found in more than 14 per cent of all persons coming to autopsy, while Kaufmann<sup>4</sup> gives the percentage as 80.

It will be noted that the sex incidence of milk spots is fairly similar, males made up 62.7 per cent of the group with spots and 69.4 per cent of the group without spots. If the subjects 18 years of age and over are taken, the figures are even closer, being 64.7 and 69.1, respectively.

3 Adam, J. G., and Nicholls, A. G. *The Principles of Pathology*, Philadelphia, Lea & Febiger, 1909, vol. 2, p. 141.

4 Kaufmann, E. *Pathology for Students and Practitioners*, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 1, p. 13.

*Number of Spots per Subject*—The number of spots found in each subject is given in table 4. It will be noted that multiple spots were somewhat more frequent than single spots. The terms in quotation marks in the table are taken from the notes made on the subjects.

*Size of Spots*—As stated previously, the milk spots included in this series were 1 cm or more in diameter in all except 6 cases. The exact number of spots of each size found cannot be given for the reason that in most of the numerous cases of multiple spots, only the size of the largest was noted. However, it is my estimate that in a large series of

TABLE 3—Incidence of Milk Spots by Age and Sex

Age Group, Yr	Number With Spots			Number Without Spots			Number in Age Group	Percentage of Age Group With Spots
	Males	Females	Total	Males	Females	Total		
Below 18	4	1	5	26	24	50	55	9.1
18-29	8	3	11	16	13	29	40	27.5
30-39	8	8	16	18	10	28	44	36.4
40-49	11	13	24	23	21	44	63	35.3
50-59	22	10	32	45	23	68	100	32.0
60-69	34	7	41	38	21	59	100	41.0
70-79	25	5	30	33	9	42	72	41.7
80 and over	6	5	11	4	0	4	15	73.4
All ages	118	52	170	203	121	324	494	34.4
18 and over	114	51	165	177	97	274	439	37.6

TABLE 4—Number of Spots per Subject

Number of Spots	Subjects
Unspecified	12
One	62
Two	15
Three or "few"	23
"Several"	30
"Numerous" or "extensive"	23
Total	170

milk spots, the sizes 1 cm, 1.5 cm, 2 cm, 2.5 cm, 3 cm and over 3 cm in diameter will comprise about 30, 30, 20, 10, 5 and 5 per cent, respectively.

*Location of Spots*—Table 5 shows that the most common location is on the anterior surface of the right ventricle. Where the spots were numerous and extensive, the largest and thickest would more often be in this area also. The table is not complete because in about 30 cases spots were merely stated to be present, and in the cases with numerous spots each location was not noted. On the other hand, frequently cases with two or three spots will have them in one location.

Summarizing, it will be seen from table 5 that spots were noted on the right side of the heart 101 times, against 35 on the left, on the anterior surface 76 times, against 27 on the posterior, and on the ventricles 116 times, as compared with 17 on the atria

*Association of Spots with Disease*—Pericardial milk spots have not been definitely associated with any certain disease or groups of diseases Tsunoda<sup>1</sup> stated that they are commonest in hypertrophied or dilated hearts, and Girsensohn<sup>2</sup> stated that in rheumatic conditions and with

TABLE 5—Location of Spots

Location	Cases
"Numerous" or "extensive"	23
Right ventricle	
Anterior (including pulmonary conus)	54
Posterior	22
Unspecified	9
Left ventricle	
Anterior	22
Posterior	5
Unspecified	7
Right atrium	16
Left atrium	1
Intrapericardial aorta	8
Venae cavae	5

TABLE 6—Incidence in Various Disease Groups

Group	With Spots		Without Spots		Total	
	No	%	No	%	No	%
All subjects 18 or more years of age	165	37.6	274	62.4	439	100.0
First group less 42 with valvular heart disease	137	34.5	280	65.5	397	100.0
First group less 26 with severe coronary sclerosis	152	36.8	261	63.2	413	100.0
First group less 121 with enlargement of the heart	107	33.7	211	66.3	318	100.0
First group less 33 with hypertension	151	37.2	255	62.8	406	100.0
Chronic valvular heart disease	28	66.7	14	33.3	42	100.0
Severe coronary sclerosis	13	50.0	13	50.0	26	100.0
Enlargement of the heart	58	47.9	63	52.1	121	100.0
Hypertensive heart disease	14	42.4	19	57.6	33	100.0

healed or recurrent inflammation in the heart they are increased, neither author gives actual figures. From table 6 it seems definite that there is an increase in the number of spots with chronic valvular heart disease (old and recurrent rheumatic, subacute bacterial and syphilitic), 66.7 per cent of the subjects with valvular heart disease showing them, as compared with 34.5 per cent for subjects without valvular disease. Increases with severe coronary disease and cardiac hypertrophy seem fairly definite, while an increase with hypertension is uncertain. The figures

<sup>5</sup> Girsensohn, H. Virchows Arch f path Anat **293** 73, 1934

are based on the 439 subjects 18 or more years of age, the differences would be slightly greater if the entire group were used. Hearts were considered enlarged when they weighed over 400 Gm in men and 350 Gm in women.

The distribution of the 42 cases of valvular disease (old and recurrent rheumatic, subacute bacterial and syphilitic) was as shown in table 7. It will be seen that the different types of lesions show about the same incidence of spots.

*Relation to Pleural Adhesions*—The presence and the degree of pleural adhesions were noted in 100 subjects with and 100 without spots, to see if there was any increase in the incidence of adhesions in the group with spots. Only old fibrous adhesions were considered. Of the 100 subjects with pericardial milk spots, 54 also had pleural adhesions, in 25 of whom the adhesions were extensive enough to involve one half or more of the total pleural surface. Of the 100 subjects without milk spots, 38 had pleural adhesions, and in 20 of these the adhesions were

TABLE 7—*Distribution of Cases of Valvular Disease with Regard to Spots*

	Mitral Valve		Aortic Valve				Other Valves
	Old or Recurrent Rheumatic Lesion	Subacute Bacterial Lesion	Old or Recurrent Rheumatic Lesion	Subacute Bacterial Lesion	Calcified Nodular Lesion	Syphilitic Lesion	Subacute Bacterial Lesion
With spots	11	3	5	1	5	2	1 (pulmonary)
Without spots	7	1	3	0	1	1	1 (tricuspid)

extensive. Thus, there appears to be a slight increase in pleural adhesions in the group with pericardial milk spots.

*Size of Heart*—The mean weight of the hearts from the 165 subjects 18 or more years of age in whom pericardial milk spots were present was 378 Gm, that of the hearts from the 274 subjects in whom pericardial milk spots were not present was 352 Gm.

*Parietal Pericardium*—Thickenings of the parietal pericardium, similar in appearance and distribution to those on the visceral pericardium, occurred frequently, although less so than the latter. No special study of the parietal thickenings was made.

#### EXPERIMENTAL PRODUCTION

This was first attempted by Tsunoda,<sup>1</sup> who introduced pyroxylin, glass or rubber foreign bodies under the sternums of dogs and rabbits (number not stated), and then examined the pericardium after intervals of one week to thirteen months. During the first two postoperative months the pericardium was essentially normal, but after four to six

months it showed cloudy areas grossly and a slight increase in collagen microscopically. white collagenous areas appeared in from six to thirteen months. Ishisaki<sup>6</sup> produced epicardial thickenings in 24 of 26 guinea pigs by constricting the thorax with a plaster cast. Most of these epicardial thickenings were slight in degree, but a few were marked. There were also thickenings of the parietal pericardium. Good gross and microscopic descriptions and illustrations are given. Guggensohn<sup>7</sup> injected horse serum intrapericardially into sensitized rabbits through an operative incision, then killed them six to thirteen weeks later. Pericardial thickenings were found in 5 of the 10 animals. Whether similar lesions might have been produced by operative intrapericardial injection with its unavoidable trauma, in unsensitized animals was not mentioned.

#### MICROSCOPIC OBSERVATIONS

Detailed microscopic descriptions of pericardial milk spots are to be found in the literature,<sup>7</sup> and there is no intention to duplicate them in this paper. I will give only a few observations from the study of 132 spots in this series which were microscopically sectioned. Though their thickest portions, 27 spots were from 100 to 200 microns thick, 76 were from 200 to 350 microns, 19 were from 350 to 500 microns, and 10 were over 500 microns thick, these are approximate measurements, made by comparing the thickness of the milk spot with a microscopic field of a known width of 440 microns. Small to large collections of lymphoid cells just underneath the fibrous spots were frequent, and fairly large numbers of lymphoid cells were present within the spots in several instances. Eleven spots showed serosal epithelium enclosed within or penetrating into the collagenous tissue in glandular or canalicular formation. Slight to moderate palisading of fibroblastic cells just under the serosal epithelium was seen in 9 cases. Long frondlike villi projecting into the pericardial cavity were present in 4 cases, and shorter villi or overhanging ends of collagenous masses were present in 11 more. Frequently more than one of these atypical features were present. These appearances, present in 15 or 20 per cent of this series of milk spots, suggest or are transitions from an inflammatory process to the usual type of milk spot. They were not seen in any particular type of case, but with all types.

#### SUMMARY

Pericardial milk spots occurred in 170 (34.4 per cent) of 494 persons 1 year or more of age. In 439 persons 18 or more years of age the incidence was 37.6 per cent. In general, there is an increase of inci-

<sup>6</sup> Ishisaki, S. *Virchows Arch f path Anat* **244** 214, 1923.

<sup>7</sup> Tsunoda<sup>1</sup> Ribbert. *Virchows Arch f path Anat* **147** 193, 1897.



dence with age, but this increase is by no means rectilinear. The spots are scarce in children and very frequent in old age, but between 35 and 75 years of age there is little change in incidence.

There seems to be a definite association with chronic or recurrent valvular heart disease, of 42 persons with such disease, 28 (66.7 per cent) showed spots. Patients with severe coronary sclerosis and enlarged hearts showed fairly definite increases (50.0 and 47.9 per cent, respectively).

The occurrence of more than one spot is slightly more frequent than that of only one. Spots occur on the right side, anteriorly, and on the ventricles much more frequently than they do on the opposite surfaces.

Old pleural adhesions are slightly more frequent in patients with spots than in those without.

Fifteen or 20 per cent of spots show appearances (projecting villi, cellular exudation, subepithelial palisading or epithelial enclosures in collagenous tissue) which suggest transitions from a more active inflammatory process to the usual type of milk spot.

# Case Reports

## MALIGNANT GRANULOSA CELL TUMOR OF THE OVARY

J S MCCARTNEY JR, M D, MINNEAPOLIS

Although a large number of granulosa cell tumors of the ovary have been reported, especially in recent years, as granulosa cell carcinoma, it is doubtful whether the name "carcinoma" should be universally applied to them. A majority of the reports deal with tumors removed at operation. Only a few of the patients have been followed for a period of five years after operation, and there are no detailed post-mortem reports on those whose death was due to metastases.

The diagnosis of malignancy has been based almost entirely on the microscopic appearances in surgical specimens. Novak<sup>1</sup> estimates the degree of malignancy as slight, with some 5 to 10 per cent recurring and metastasizing, a few being highly malignant. Meyer<sup>2</sup> in following up 33 patients found that 3 had died from metastases, but he gave no details. Habbe<sup>3</sup> in following up Meyer's 33 patients found that there had been 5 deaths (2 postoperative) and 4 recurrences. Klasten<sup>4</sup> reported that in a group of 80 collected instances recurrences or metastases were present in 4, i e., 5 per cent. Novak and Brawner<sup>5</sup> stated that of 32 patients, 6 showed unmistakable signs of a malignant growth at the time of operation and 3 had recurrences and that therefore 28.1 per cent of the growths were malignant. Schiller<sup>6</sup> in a study of 24 granulosa cell tumors concluded that from the microscopic standpoint 8 were malignant. Novak and Gray,<sup>7</sup> studying 42 tumors, found that the degree of malignancy as estimated by microscopic observation was less than in ovarian carcinomas as a whole. Taussig<sup>8</sup> stated that such tumors were usually unilateral and showed no tendency to metastasize.

Von Werdt<sup>9</sup> reported an instance in which in a patient who died three and one-half months after operation no metastases were found at necropsy. Voigt's<sup>10</sup> patient died about six months after operation with evidences of recurrence, but no autopsy was made. One of Neu-

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From the Department of Pathology, University of Minnesota

1 Novak, E. *Am J Obst & Gynec* **26** 505, 1933. Novak, E., and Long, J. H. *J A M A* **101** 1057, 1933.

2 Meyer, R. *Arch f Gynak* **145** 2, 1931.

3 Habbe, E. *Zentralbl f Gynak* **55** 1088, 1931.

4 Klasten, E. *Arch f Gynak* **150** 643, 1932.

5 Novak, E., and Brawner, J. N. *Am J Obst & Gynec* **28** 637, 1934.

6 Schiller, W. *Pathologie und Klinik der Granulosazelltumoren*, Vienna, Wilhelm Maudrich, 1934.

7 Novak, E., and Gray, L. A. *Am J Obst & Gynec* **31** 213, 1936.

8 Taussig, F. J. *Am J Cancer* **15** 1547, 1931.

9 von Werdt, F. *Beitr z path Anat u z allg Path* **59** 453, 1914.

10 Voigt, M. *Arch f Gynak* **70** 87, 1903.

mann's<sup>11</sup> patients died six months after operation with recurrence of the tumor. Aschner's<sup>12</sup> patient also died about six months postoperatively with signs of recurrence, but there was no postmortem examination.

Arnold, Koerner and Mathias<sup>13</sup> reported the following case. A woman 68 years of age, with a granulosa cell tumor, was first operated on for an ovarian tumor in 1908. She had an incomplete operation for the removal of a recurrence in 1924 and finally died in 1928 of "carcinosis universalis."

Te Linde<sup>14</sup> searched the literature for the end results in granulosa cell tumor and found that of 17 patients followed, 13 were reported as well for from one to eleven years after operation. Of these 13, 2 only were followed for as long as five years—one, reported by Isbruch,<sup>15</sup> was a woman aged 50 years, who was still alive and well eleven years later, and the other, reported by Mullerheim,<sup>16</sup> was a woman aged 69 years, who was still well eight years after the operation.

Schiller included 16 granulosa cell tumors in his benign group. Of the patients, 4 were alive and well five years, 1 nine years and 1 ten years after operation. Another died one month after operation, of pulmonary embolism, and at postmortem examination had metastases in the left kidney.

A third case of survival for more than five years may be added. This concerned a woman aged 20 years who was operated on on May 6, 1932, and an ovary partially replaced by a tumor 2 cm in diameter removed. This tumor was in part typically follicular and in part cylindroid. The patient was alive and well, without evidence of recurrence, on Nov 15, 1938.

Fauvet<sup>17</sup> reported 8 instances of granulosa cell tumor. One patient died postoperatively of pulmonary embolism, and it was found that the ovarian tumor had perforated its capsule and spread regionally, in another patient recurrence took place. Soltmann's<sup>18</sup> patient died ten days after operation from paralytic ileus, metastases were present in the sacrum. Schulze<sup>19</sup> reported 4 instances of granulosa cell tumor and mentioned that in 1 instance death with recurrence took place four years after operation, autopsy was apparently not done.

Of the 8 patients with malignant tumors reported by Schiller and mentioned in a foregoing paragraph, 2 showed metastases at the time of operation, a third died four days after operation from pulmonary embolism and showed at postmortem examination peritoneal metastases, while a fourth died four months after operation with generalized metastases. On the last an autopsy was apparently not done.

11 Neumann, H. O. *Virchows Arch f path Anat* **258** 284, 1925.

12 Aschner, B. *Arch f Gynak* **115** 350, 1922.

13 Arnold, W., Koerner, J., and Mathias, E. *Virchows Arch f path Anat* **277** 48, 1930.

14 Te Linde, R. W. *Am J Obst & Gynec* **20** 552, 1930.

15 Isbruch, F. *Zentralbl f Gynak* **50** 89, 1926.

16 Mullerheim, R. *Zentralbl f Gynak* **52** 689, 1928.

17 Fauvet, E. *Zentralbl f Gynak* **56** 3088, 1932.

18 Soltmann, C. H. *Virchows Arch f path Anat* **284** 466, 1932.

19 Schulze, M. *Am J Obst & Gynec* **26** 627, 1933.

Since there have been so few instances of this disease reported in which the final outcome was ascertained by autopsy it seems worth while to place such a case on record

#### REPORT OF A CASE

Uterine curettings from a woman aged 59 years were submitted for examination, Feb 19, 1932, and a diagnosis was made of hypertrophic endometrium (fig 1A) No history was obtained at that time

August 19 specimens of uterus and ovarian tumor were sent for examination On this occasion the following history was obtained The patient's mother died of cancer of the breast The patient had borne 2 children The menopause took place in 1918 (fourteen years before) There was no subsequent bleeding until about one year before the last admittance to the hospital The bleeding was entirely without regularity, at times there was spotting every four or five days, and there were periods as long as eight weeks with no hemorrhage At times many large clots were passed The most severe of all the hemorrhages had taken place August 16 On admission on that day the patient complained of a sharp pain radiating down the left leg She had lost about 16 pounds (7.3 Kg) in weight There was the additional history of the removal of a cervical polyp about ten months before, curettage had been done the preceding February, followed with treatment by radium Examination showed an obese woman with distinct evidences of anemia The hemoglobin content was 45 per cent, the red blood cells, 3,230,000 The blood pressure ranged between 180 systolic and 90 diastolic and 154 systolic and 82 diastolic The cervix was lacerated and cystic

At operation on August 19 the left ovary contained a hemorrhagic cyst, about 20 cm in diameter, the uterus was about twice the normal size, with a fibrous myometrium and many myomas The endometrium was irregularly thickened Subtotal hysterectomy and left salpingo-oophorectomy were done

Sections of the ovary showed numerous cysts, most of them with no epithelial lining There were large areas of necrosis and old and fresh hemorrhages Some remnants of ovarian tissue were present along one edge The relatively solid portion of the tumor was made up of solid cellular areas, the cells of which in places assumed a cordlike arrangement In places small rounded spaces were present, which resembled follicles The cells stained fairly uniformly but were of a variety of shapes polyhedral, rounded, elongated and flattened Scattered through the tumor were fibrous trabeculae, usually hyaline, which did not divide the tumor cells into nests Mitotic figures were not found (fig 1B) Sections of the uterus showed adenomyoma There was cystic, hypertrophic endometrium A diagnosis was made of carcinoma of the ovary, with the suggestion that it was of the granulosa cell type

The patient was not seen again until May 27, 1937, at which time she came under the care of another physician She complained of a sensation of fulness in the epigastrium, constipation and edema of the ankles She stated that following the operation she was in good health until about one year before, when she noted swelling of the ankles In December 1936 she had "influenza" with vomiting, headache and general malaise Since that time she had had the sensation of fulness in the upper part of the abdomen, it bore no relation to meals There had been no loss of weight Physical examination revealed pitting edema of the ankles and a large, freely movable, apparently cystic mass, about the size of a full term pregnancy, filling the abdomen At operation a very large multilocular cystic tumor, apparently arising from the right ovary and adherent to the adjacent



Fig 1—*A*, cystic, hyperplastic endometrium, removed Feb 19, 1932 *B*, low power photomicrograph of the right ovarian tumor, removed Aug 19, 1932

structures, was found. The adhesions were not dense. A number of other small cysts were also present, but the general peritoneal surfaces seemed not to be involved. The entire mass was removed. A portion of it was sent for examination. This showed numerous large cysts and some solid areas.

The sections showed a tumor somewhat resembling that found in the left ovary but not so well differentiated. There were roughly rounded oval cellular areas, separated from one another by narrow and broad masses of loose tissue, somewhat resembling areolar connective tissue. The cellular masses were not sharply bounded and seemed to fade into the other type of tissue. Higher magnification showed that the cellular areas were composed of fairly uniform cells, indistinctly arranged in cords. Occasional structures suggesting follicles were observed. No mitotic figures were found. There were no glands or recognizable ovarian tissue (fig 2A). A diagnosis was made of carcinoma of the ovary.

The patient was next seen July 10, 1938, when the history was obtained that after the second operation she felt well until the preceding winter, when weakness of the left leg and spells of dizziness were noted. She complained of pain in the right side of the head and difficulty in hearing in the right ear. During recent weeks there had been spells of a projectile type of vomiting and abdominal pain. Polyphagia was also complained of. She seemed to understand what was said to her but either would not or could not answer. The blood pressure at this time was 190 systolic and 100 diastolic. Some fever was present, and in succeeding days the temperature varied from 104 to 105.5 F. Death occurred July 15. The age at this time was 65 years.

Postmortem examination, done twelve hours after death, showed a well nourished white woman. Two old scars were present on the abdominal wall but no evidences of tumor in either. There was edema of the ankles and over the sacrum. The abdominal fat was 5 cm in thickness. Scattered fibrous adhesions were present in the peritoneal cavity. The appendix was small, fibrous and nonadherent. Throughout the peritoneal cavity were brownish red to gray nodules, of fleshy consistence and varying from 0.5 to 2 cm in diameter. They were present on the parietal and visceral layers of the peritoneum and in the mesentery. Attached to the posterior parietal peritoneum between the liver and the spine was a firm, grayish brown pedunculated mass, 2 by 3 cm.

The heart weighed 400 Gm and was normal except for some myocardial fibrosis. The lungs showed a small amount of purulent bronchitis and bronchopneumonia. The spleen was of normal size. The liver disclosed evidences of chronic passive congestion. The gallbladder, gastrointestinal tract, pancreas and adrenals were normal. The kidneys had finely granular surfaces.

The pelvic structures showed no evidences of tumor. The stump of the cervix was 2.5 cm in diameter and contained a single cyst, filled with gelatinous material.

An encapsulated nodule, 1 cm in diameter, was present in the right breast. A calcified mass, 3 cm in diameter, was present in the left breast.

The scalp, calvarium and meninges appeared normal. Cross sections of the brain showed that the anterior portion of the right lateral ventricle was almost completely filled by a reddish gray, soft, well outlined, partially necrotic mass, 3.5 by 2.75 by 2.75 cm. It involved the lower portion of the corpus callosum and protruded into the left lateral ventricle. The right caudate nucleus and the superior aspect of the right internal capsule were also involved. In the anterior portion of the left parietal lobe was a firm gray nodule about 7 mm in diameter. The microscopic observations were as follows. The mesenteric lymph node showed the normal

tissue entirely replaced by tumor tissue, which for the most part was composed of irregular islands having a distinct cordlike arrangement (fig 2 *B*). The diffusely cellular parts had appearances much like those of the tissue removed at the second

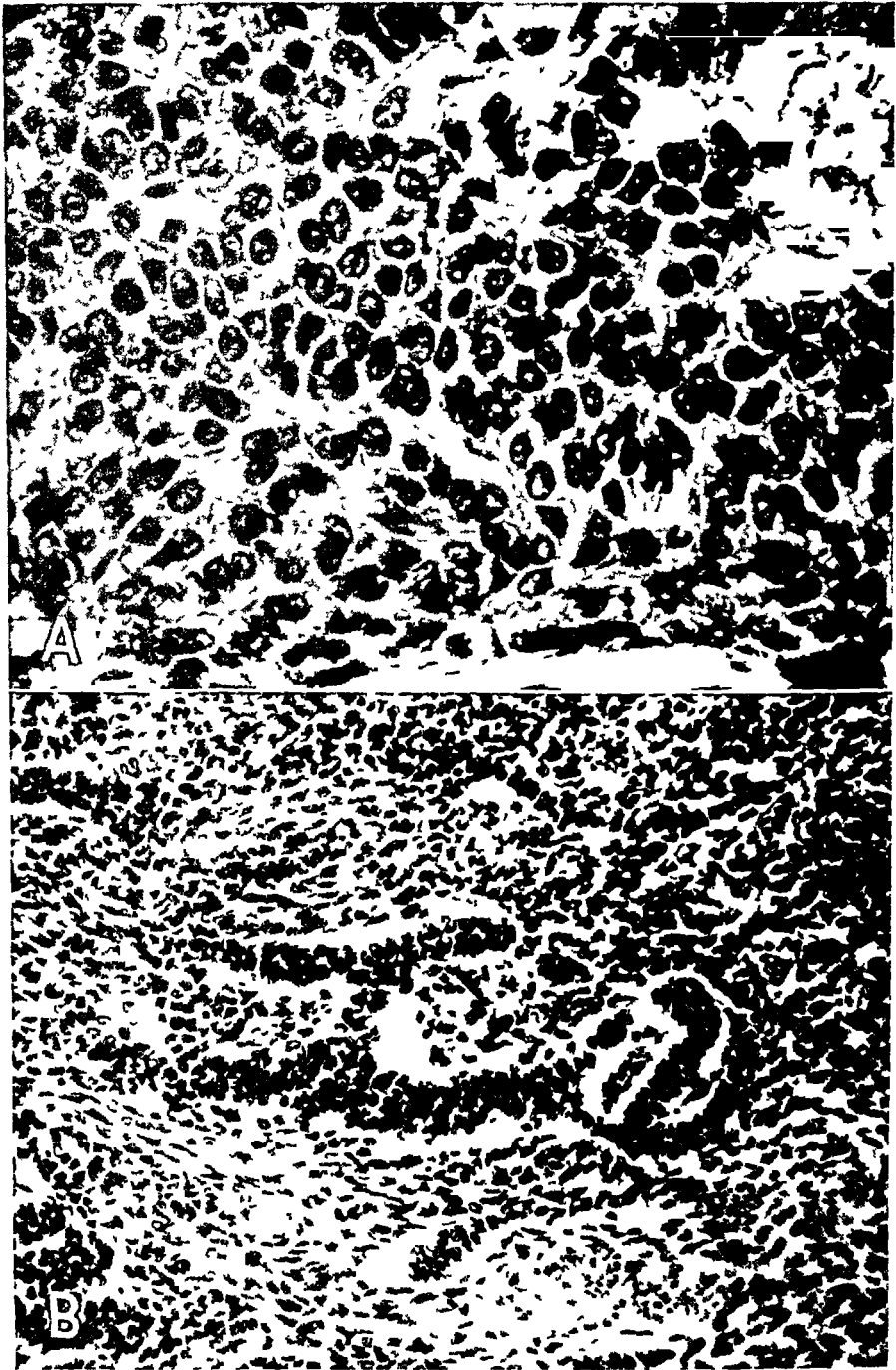


Fig 2—*A*, high power photomicrograph of the tumor of the left ovary, removed in May 1937. *B*, high power photomicrograph of a metastasis in a lymph node. The normal structure of the node is entirely replaced by tumor, partly in the form of diffuse cellular sarcoma-like tissue, partly in the form of twisted cords.

laparotomy, but with no cysts. Structures suggesting follicles were occasionally found. No mitotic figures were present.

The peritoneum structurally took the form of twisted cords of cells separated by spaces somewhat suggestive of gland spaces (fig. 3*A*).

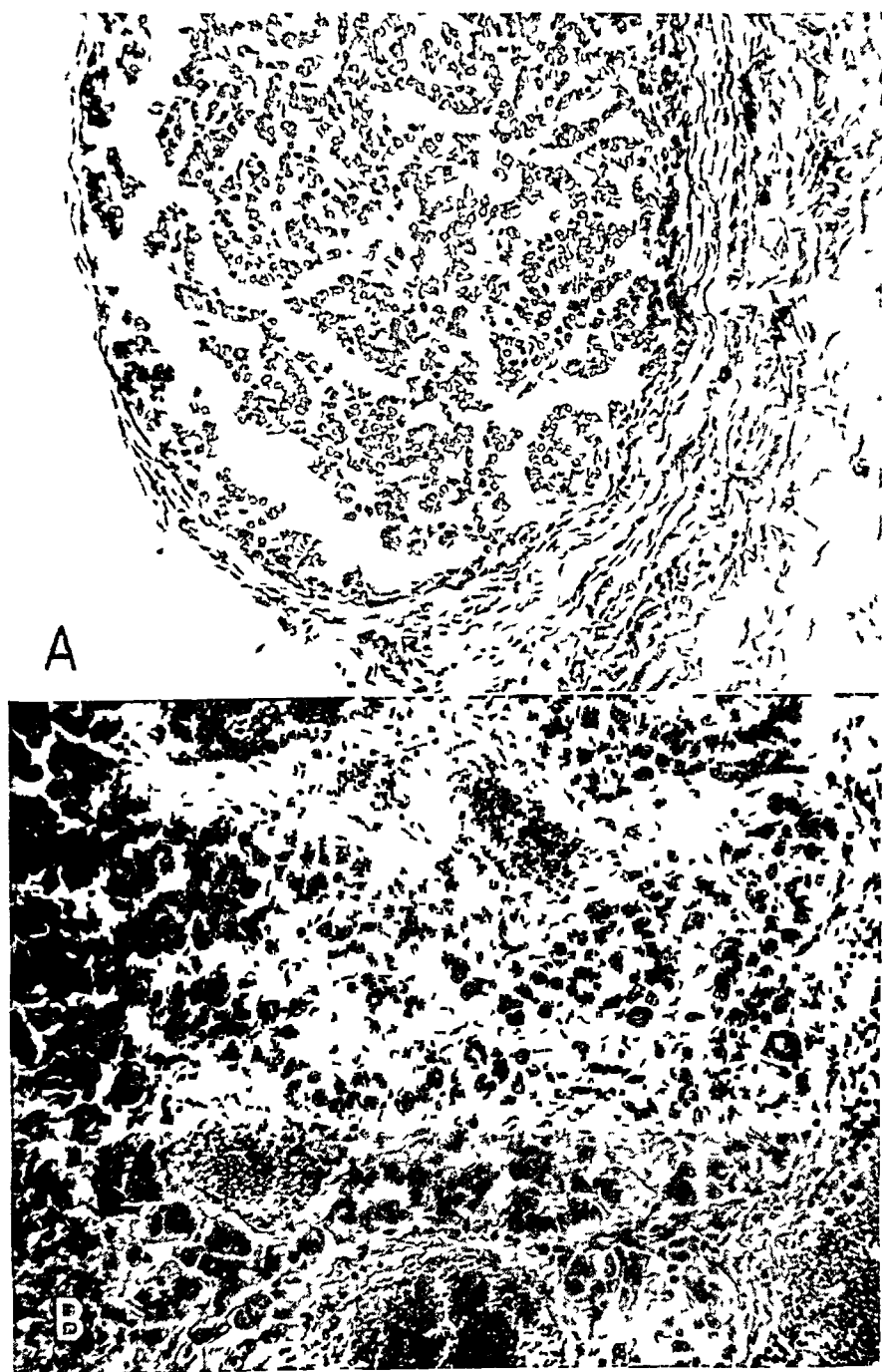


Fig. 3—*A*, photomicrograph of a peritoneal metastasis. Note the cylindroid arrangement of the cells in twisted cords. *B*, photomicrograph of a metastasis in the brain. Note the large cells in the form of solid cords with only a slight tendency toward follicle formation.



Sections from the two tumors in the brain showed appearances which did not very closely resemble one another or those of the tumors elsewhere in the body. They showed a very great polymorphism of the cells with rather numerous multinucleated giant cells. One section (fig 3B) disclosed large cordlike masses of rather large polyhedral cells with, in some areas, appearances vaguely reminding one of follicles. The other tumor was made up of a tissue having a rather fibrous structure with comparatively small and scattered cells. A number of giant cells were present, some of which appeared like rosettes. Follicle-like structures were hard to find, unless the rosettes are to be considered follicles.

The tumors of the breasts were found to be fibroadenoma.

The origin of granulosa cell tumors has been discussed by many authors, and the majority accept Meyer's opinion that they come from cell nests and not from adult granulosa cells. Meyer recognizes three types, folliculoid, cylindroid and diffuse, the last resembling sarcoma, all of which may coexist in the same tumor. There are also numerous papers dealing with the physiologic effects of the secretion of these tumors. During sexual life there may be amenorrhea or irregular menstruation. The usual effect after the menopause is a recurrence of uterine bleeding. Such tumors developing in young children cause precocious puberty.

The case reported is an instance of a granulosa cell tumor developing about fourteen years after the menopause. It produced uterine bleeding and a typical hyperplastic, cystic endometrium. Following removal of the ovary which contained the tumor the patient was well for a period of almost five years. At this time another tumor developed in the opposite ovary. This second tumor also had the structure of a granulosa cell tumor but was not quite identical with the first. It is probable that the second tumor represents a metastasis from the first. Although the granulosa cell tumor is usually unilateral, several authors have reported examples of a bilateral tumor (Rummeld,<sup>20</sup> Klasten<sup>4</sup>). Following removal of the second ovarian tumor the patient had a period of good health, which lasted for only a few months, after which symptoms of an intracranial lesion developed. Post mortem metastases were found in the peritoneum, the mesenteric lymph nodes and the brain.

#### SUMMARY

A granulosa cell carcinoma of the ovary is reported, which developed fourteen years after the menopause and produced cystic hyperplasia of the endometrium with uterine bleeding. Five years after removal of the tumor there appeared signs of involvement of the opposite ovary. This was also removed. Death occurred one year later or six years after the first operation. Postmortem metastases were found in the mesenteric lymph nodes, the peritoneum and the brain.

An instance of a patient alive and well six and one-half years after removal of a granulosa cell tumor is also reported.

The diagnosis of granulosa cell carcinoma should be reserved for the tumors of granulosa cell type which are definitely malignant.

# SYMMETRIC NECROSIS OF THE GLOBUS PALLIDUS IN BARBITURATE POISONING

A. DR. GROAT, M.D., DETROIT

Necrosis in the basal ganglions from intoxication is commonly produced by no agent other than carbon monoxide. This is remarkable in view of the fact that carbon monoxide probably acts only through anoxemia. Asphyxia from other causes, including depressant drugs, shows no such selective action on the basal ganglions, rather it produces diffuse lesions both in man and in experimental animals.

However, Gonzales, Vance and Helpern<sup>1</sup> reported a case of symmetric softening of the globus pallidus due to barbital poisoning which seems to be unique in the literature. I have recently encountered a similar case which is reported here for its theoretic and medicolegal interest.

## REPORT OF A CASE

The subject was a white woman 24 years old, a nurse, apparently in good health. She had attended a social function and had retired at a normal hour. The next morning she was discovered by her roommate to be unconscious. There were marked cyanosis and feeble respiration. The cyanosis deepened, and the breathing became irregular, necessitating artificial respiration at intervals over a period of about six hours. The reflexes were absent but returned slowly over a period of about two days. The patient remained unconscious until her death five days later, from bronchopneumonia.

From a further investigation of her history it was apparent that she was addicted to a stupefactive of some kind, and pentobarbital sodium was found among her personal effects.

At necropsy the chief anatomic changes were edema of the brain, symmetric softening of the globus pallidus, bronchopneumonia and fatty degeneration of the liver. There was a small amount of calcification in the arterioles of the corpus striatum, and degeneration of ganglion cells of varying degrees was found throughout the brain.

On the basis of what is known of the toxic properties and effects of carbon monoxide one may conclude that necrosis of the globus pallidus occurs when there is prolonged deep asphyxia followed by temporary survival for several days. It thus appears that the same lesion may result from the action of barbiturates when these chance to produce the same set of circumstances. It is even conceivable that the lesion might follow strangulation, nitrogen monoxide anesthesia, the syncope of Stokes-Adams disease and irritation of the carotid sinus. An alternative theory is that carbon monoxide and the barbiturates exert a specific effect on the globus pallidus, but for this there is little evidence.

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<sup>1</sup> Gonzales, T. A., Vance, M., and Helpern, M. Legal Medicine and Toxicology, New York, D. Appleton-Century Company, Inc., 1937.

# Laboratory Methods and Technical Notes

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## A MODIFICATION OF MASSON'S TETRACHROME STAIN

For Routine Paraffin Sections of Tissue Fixed in Solution of Formaldehyde and Saline Solution

CHARLES P. LARSON, M.D., C.M., TACOMA, WASH.  
AND E. J. LEVIN, SOAP LAKE, WASH.

During the past year trichrome and tetrachrome stains have been adopted by many laboratories as routine stains for paraffin sections. This has been due largely to the wide acceptance of Goldner's<sup>1</sup> modification of the Masson<sup>2</sup> stain. The original Masson trichrome stain still gives the most precise cytoplasmic and nuclear detail, but because of the length of time required for staining, the special fixation and the necessity of handling sections individually, it cannot be adopted by most pathologists for routine work.

The modification described here allows for the use of tissue fixed in solution of formaldehyde-saline solution and is very similar to the method of Goldner, but in view of the superior results which we have secured with slight alterations of his technic we feel justified in publishing our routine. Without counting the time required for deparaffination, the staining of a slide with this technic requires sixty minutes. Trays of 30 or more slides may be stained at one time with no individual handling and with surprisingly uniform results.

This stain has proved to be exceptionally good for all routine surgical and autopsy material and has the following advantages not possessed by hematoxylin and eosin. It differentiates every muscle fiber from connective tissue, it differentiates nerve tissue, it gives unusually clear detail in neoplastic growths, and as a routine survey stain for the central nervous system it is eminently satisfactory, as it brings out neuronal details and demarcates demyelinations. However, a word of caution may be given to those unaccustomed to the use of trichrome stains. There may be difficulty in evaluating the degree and extent of simple exudative and granulomatous lesions. With a little experience in interpreting the stain this difficulty will be overcome.

The stain may also be used on material fixed in Zenker's solution, provided the usual routine following the use of this fixative is adhered to, i. e., with iodine and thiosulfate and steps 3 and 4 in the routine left out.

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From the Tacoma General Hospital, Tacoma (Dr. Larson), and McKay Memorial Research Hospital, Soap Lake, Wash.

1 Goldner, J. *Am J Path* **14** 237, 1938.

2 Masson, P. *J Tech Methods* **12** 75, 1929.

# PROCEDURE

- 1 Deparaffinize in xylene
- 2 Hydrate by bringing down through graded alcohols
- 3 Mordant for ten minutes in 5 per cent potassium dichromate
- 4 Wash in running tap water for ten minutes
- 5 Stain ten to fifteen minutes in Hansen's trioxhematein
- 6 Wash five minutes in running tap water
- 7 Wash one minute in 1 per cent glacial acetic acid
- 8 Stain five minutes in ponceau-fuchsin stain
- 9 Wash two minutes in 1 per cent glacial acetic acid
- 10 Differentiate one minute in 2 per cent phosphotungstic acid
- 11 Wash two minutes in 1 per cent glacial acetic acid
- 12 Stain in light green mixture for five minutes
- 13 Wash for three minutes in 1 per cent glacial acetic acid
- 14 Dehydrate in two changes of 95 per cent alcohol
- 15 Clear in carbolxylene
- 16 Mount in Canada balsam or dammar

## FORMULAS FOR PREPARATION OF REAGENTS

- 1 Alcohols may be ethyl or isopropanol (anhydrous)

- 2 Hansen's trioxhematein is prepared as follows

Solution A Dissolve 10 Gm of ammonium-ferric alum and 1.4 Gm of ammonium sulfate in 150 cc of distilled water with heat

Solution B Dissolve 1.6 Gm of hematoxylin in 75 cc of distilled water with heat

Thoroughly cool both solutions. Then pour solution A into solution B (never vice versa), stirring constantly. When the mixture turns violet, heat it over flame and test it on filter paper for a sepia or a brownish black color. Remove the solution from flame immediately and cool by immersing the beaker in cold water. If the stain shows an olive green color on the filter paper, it is overoxidized. Do not boil the stain longer than one minute.

Dilute the prepared stain with an equal amount of 1 per cent sulfuric acid. Store in a stoppered bottle filled to the constricted portion, which exposes only a small area to oxidation. Filter each time before use.

The stain is stable for from four to six weeks.

- 3 The ponceau-fuchsin stain is made up as follows

Stock Dissolve 1.5 Gm of ponceau de xylidine and 0.5 Gm of acid fuchsin in 200 cc of 1 per cent glacial acetic acid

Staining solution 10 cc of stock and 2 cc of 0.5 per cent aqueous azophloxin mixed with 88 cc of 1 per cent glacial acetic acid

- 4 Light green is prepared as follows

Stock Dissolve 0.2 Gm of light green S F in 100 cc of 1 per cent glacial acetic acid solution

Staining solution 20 cc of stock to 80 cc of 1 per cent glacial acetic acid solution

- 5 Carbolxylene is made up of 3 parts xylene to 1 part chemically pure phenol

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—In recognition of his work in the field of cancer research, James B Murphy, a member of the Rockefeller Institute for Medical Research, has received the decoration of Officer of the Order of Leopold from the king of Belgium

Baxter L Crawford, assistant professor of pathology in Jefferson Medical College, died Jan 3, 1940, 53 years old

In New York Medical College, L Corsan Reid has been promoted to the position of associate professor, and F D Spear to that of assistant professor of pathology

Francis Carter Wood, director of the Institute of Cancer Research of Columbia University, has been awarded the gold medal of the Radiological Society of North America in recognition of his achievements in the science of radiology

Olan I Cutler, professor of pathology in the College of Medical Evangelists, Loma Linda, Los Angeles, died as the result of an accident

**Fellowship in Pathology**—A research fellowship in the laboratory of pathology at the Collis P Huntington Memorial Hospital, Boston, and in the department of pathology at the Harvard Medical School will be available September 1. According to *Science*, it carries a stipend of \$3,000 and may be renewed for a second year. The fellow will be expected to devote most of his time to histologic and cytologic studies of the effects of radiation of different types on normal and pathologic tissue. Applications should be addressed to Dr Shields Warren at the Collis P Huntington Memorial Hospital, Boston

**Society News**—The American Association for the Study of Goiter will hold its next meeting at Rochester, Minn., April 15, 16 and 17, 1940. The Van Meter Prize Award for the best essay on original work on thyroid problems will be made at this meeting

Maurice N Richter has been elected president of the New York Pathological Society, Jean Oliver, vice president, and D Murray Angevine, secretary

A F Blakeslee, of the Carnegie Institution of Washington, has been elected president, and Paul R Cannon, of the University of Chicago, vice president and chairman, of the section on medical sciences of the American Association for the Advancement of Science

The next annual meeting of the American Society of Clinical Pathologists will be held at the Biltmore Hotel, New York, June 7, 8 and 9, 1940

The annual meeting of the American Association of Pathologists and Bacteriologists will be held at the Mellon Institute of Industrial Research, Pittsburgh, March 21 and 22, 1940. A symposium will be held on the pathologic aspects of vitamin deficiencies

The Pathological Society of Philadelphia has elected the following officers: president, Jefferson H Clark, vice president, R Philip Custer, and secretary-treasurer, Herbert L Ratcliffe

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

HISTOLOGIC CHANGES IN THE PERIPHERAL NERVES OF THE RAT IN VITAMIN B<sub>1</sub> DEFICIENCY C O PRICKETT, W D SALMON and G A SCHRADER, Am J Path **15** 251, 1939

The histologic changes in the peripheral nerves of rats suffering from either acute or chronic vitamin B<sub>1</sub> deficiency have been studied by the polarized light method and the sudan III method. The nerves of rats that had symptoms of acute deficiency showed only a few fibers with wallerian degeneration, such as may be seen in normal nerves, and mild edema. The nerves of control rats, which received adequate amounts of vitamin B<sub>1</sub> but with the food intake limited to that of the deficient rats, showed mild edema and more wallerian degeneration than was found in the nerves of the deficient rats. Nerves of deficient rats that became moribund without the development of typical neuromuscular symptoms showed more wallerian degeneration than those in which neuromuscular symptoms developed, but less than the control rats receiving adequate vitamin B<sub>1</sub> with a limited food intake. Residual symptoms, consisting of disordered equilibration or paralysis, occurred in rats in which a state of chronic deficiency had been produced by the administration of small amounts of thiamin chloride after the development of neuromuscular symptoms. The chronic deficiency produced marked changes of an edematous type in the peripheral nerves. The fibers were enlarged, in some cases showing large bulbous areas along their course, and contained increased amounts of isotropic material, in the most severe cases some fibers were completely isotropic. These changes were demonstrable by the polarized light method but not by the sudan III method. The rats eventually reached a stage at which cures were no longer possible even by the administration of relatively large doses of thiamin chloride. The changes in the nerves indicated that this was the result of irreparable damage to the tissues

FROM AUTHORS' SUMMARY

FAT TISSUE IN EXPERIMENTAL EXOPHTHALMOS G K SMITSER, Am J Path **15** 341, 1939

The injection of an extract of the anterior lobe of the pituitary gland produced exophthalmos and marked edema of the orbital fat and connective tissue. Fat tissues in various parts of the body were affected by the injection of the anterior pituitary extract but to a lesser degree than that in the orbit. The difference in the degree of edema produced in the orbit and in other fat tissues may have been due to the difference in structure of the orbital fat tissue. The edematous material found in the orbit appeared to be identical in composition with that found elsewhere in the same animal.

FROM AUTHOR'S SUMMARY

RENAL FUNCTION AND THE NUMBER OF GLOMERULI IN THE HUMAN KIDNEY J M HAYMAN JR, J W MARTIN JR and M MILLER, Arch Int Med **64** 69, 1939

The clearance of creatinine and urea, the maximum specific gravity of the urine and the blood pressure were correlated with the number of glomeruli per kidney (estimated after postmortem perfusion) for patients with and for patients without disease of the kidneys. In those with chronic glomerulonephritis and

nephrosclerosis the creatinine and urea clearance were closely correlated with the number of glomeruli. The maximum specific gravity of the urine falls with decrease in the number of glomeruli until the latter reaches 700,000 to 800,000 per kidney, after which it remains fixed in spite of further reduction in the number of glomeruli. If the number of glomeruli per kidney is less than 700,000, the systolic blood pressure is invariably above 150 mm. In certain patients with acute infections and jaundice the urea and creatinine clearance and the concentrating ability were both markedly reduced in spite of a normal number of glomeruli showing no significant change in histologic sections.

FROM AUTHORS' SUMMARY

ETIOLOGIC FACTORS IN EXPERIMENTALLY PRODUCED PONTINE HEMORRHAGES L. V. DILL and C. E. ISENHOUR, Arch Neurol & Psychiat **41** 1146, 1939

Space-consuming intracranial lesions are occasionally associated with hemorrhages in the pons. In the authors' case, for instance, a subdural hematoma and a hemorrhage in the island of Reil were combined with multiple fresh hemorrhages in the pons. The authors see the cause of the pontile hemorrhages in the pressure exerted by the subdural hematoma on the brain and the forcing down (herniation) of the pons into the foramen magnum, which caused disruption of the parenchyma. They were able to produce pontile hemorrhages in dogs by increasing the intracranial pressure (by repeated inflation of a rubber balloon placed over the animal's parietal cortex). Hemorrhages (perivenous and periaxial) were produced in the pons, medulla and occasionally in the cerebellum and basal ganglia. Anoxemia as an etiologic factor was ruled out.

GEORGE B. HASSIN

EXPERIMENTAL HYPERTENSION BY CONSTRICTION OF THE AORTA H. GOLDBLATT, J. R. KAHN and R. F. HANZAL, J. Exper Med **69** 649, 1939

Constriction of the abdominal aorta just above the site of origin of both main renal arteries has little or no immediate effect on the blood pressure above the site of the clamp (carotid systolic or mean pressure), but after about twenty-four hours hypertension develops. Below the site of the clamp the immediate effect is a lowering of the femoral mean pressure. As the carotid systolic pressure becomes elevated, the femoral mean pressure also begins to rise and in some animals eventually reaches a level higher than normal, despite great constriction or even occlusion of the abdominal aorta. Constriction of the aorta just below the origin of both main renal arteries has no significant effect on the blood pressure (carotid systolic or mean pressure) above the site of the clamp. Below the site of the clamp the blood pressure falls and tends to remain down or at most returns only to the preoperative level. The uremic, convulsive (malignant or eclamptic) phase of hypertension, with renal excretory insufficiency and degenerative, necrotizing and inflammatory arteriolar lesions in many organs, has been produced by suddenly constricting to a great degree the abdominal aorta just above the origin of both main renal arteries. The renal excretory insufficiency in the animals in which hypertension develops is directly dependent on the degree of constriction of the abdominal aorta and especially on the rapidity with which constriction is produced. Hypertension following the constriction of the abdominal aorta just above the origin of both main renal arteries, whether or not accompanied by renal excretory insufficiency, is of renal origin.

FROM AUTHORS' SUMMARY

CATAPHORETIC EXPERIMENTS WITH SENSITIZED ERYTHROCYTES C. G. ANDERSON and T. J. MACKIE, Brit J Exper Path **20** 270, 1939

Anderson and Mackie have confirmed again the hemolytic effect of colloidal silicic acid and tannic acid.

EXPERIMENTAL ACUTE PIRCHIORIDIL INTOXICATION G L MONTGOMERY, Brit J  
Exper Path **20** 316, 1939

Montgomery studied the immediate effect of small intravenous doses of mercuric chloride in rabbits and found that, although there was no microscopic evidence of renal damage in the two hours of the experimental period, the injections produced circulatory collapse and proteinuria with a fall in the blood serum protein within fifteen minutes. The proteinuria quantitatively always exceeded the loss of serum protein, and the results suggest that the latter is replaced continuously from the tissue depots. Intravenously injected saline solution had no effect on the shock syndrome.

CARBON TETRACHLORIDE CIRRHOSIS OF THE LIVER E G WHITE, J Path & Bact  
**49** 95, 1939

In pigs weighing 7 to 17 Kg and aged 25 to 66 days the subcutaneous injection of a single dose of carbon tetrachloride of 0.6 cc per kilogram of body weight seldom caused clinical disturbances, yet there was necrosis of the central half of each hepatic lobule. Repair was complete by the end of a week. Some animals, however, will not tolerate this dose, and an amount as small as 0.2 cc per kilogram has caused death within twelve hours. The histologic changes occurring in the liver at intervals after a single dose are described. Repeated administration of carbon tetrachloride at four to five day intervals leads to cirrhosis which is characterized by a subdivision of hepatic lobules into small groups of liver cells ("pseudolobules") by collagen and precollagenous reticulum. The genesis of the process has been studied by examining samples of liver removed at intervals and finally by carrying out an autopsy. The most severe cirrhosis resulted from the heaviest dose, thirty-six injections of 0.2 cc per kilogram of body weight, less severe changes were produced by twenty-eight doses of 0.1 cc per kilogram or twenty-six doses of 0.05 cc per kilogram. There was no ascites, icterus or splenomegaly. The experimentally produced cirrhosis appears to resemble the naturally occurring lesion in the pig.

FROM AUTHOR'S SUMMARY

RELATION OF THROMBOSIS, EMBOLISM AND APOPLEXY TO THE WEATHER P H  
KAYSER, Virchows Arch f path Anat **302** 210, 1938

In 253 cases of thrombosis, fulminant embolism and apoplexy, in which the condition was identified at autopsy, the onset of the vascular accident was correlated with the weather conditions at the time. Kayser concludes that his material reveals a relation between the state of the weather and the onset of thrombosis and apoplexy, these diseases, which he terms meteorotropic, occurring more often during warm fronts than during cold fronts. Thrombosis, embolism and apoplexy do not occur during periods of uniform weather, during such periods deaths from carcinoma and infectious diseases are more numerous. A seasonal variation of the vascular diseases studied was not observed. In regions with a continental climate thrombosis is rarely seen. Patients susceptible to meteorologic environment react abnormally to every change in the weather. The electronic effect of the atmosphere is held to be the most active factor in the action of weather, such an effect, working through the skin on the vegetative nervous system, causes slowing of the circulation, which favors thrombosis. Petersen's monumental work on meteorologic pathologic changes is not included in the bibliography of 31 titles.

O T SCHULTZ

EFFECT OF TRANSPLANTATION OF LIVER INTO MICE PREVIOUSLY TREATED WITH  
LIVER A SYMEONIDIS, Virchows Arch f path Anat **302** 443, 1938

The subcutaneous homologous transplantation of liver into mice previously given injections of an emulsion of liver was followed by a more intense and more



rapidly developing inflammatory reaction than was observed in the controls. Encapsulation of the implant was more rapid, and necrosis more complete. The surrounding granulation tissue was less highly vascularized than in the controls.

O T SCHULTZ

### Pathologic Anatomy

THE LYMPHOID NODULES OF HUMAN BONE MARROW R J WILLIAMS, *Am J Path* **15** 377, 1939

No pathologic significance can be attached to the presence in the marrow of lymphoid nodules of the type, size and number described in this report. The evidence appears to justify the concept that the lymphoid nodules are essentially normal, though perhaps variable, constituents of the active red marrow of adults.

FROM AUTHOR'S SUMMARY

CARDIAC SEQUELAE OF EMBOLISM OF THE PULMONARY ARTERY H HORN, S DACK and C K FRIEDBERG, *Arch Int Med* **64** 296, 1939

A group of 42 cases of embolism of the pulmonary artery has been studied, in 8 of which recent structural changes in the myocardium ordinarily resulting from acute myocardial ischemia were revealed. The factors necessary for the production of such myocardial changes are discussed. These are shock, asphyxia and exaggerated vagal reflexes resulting from obstruction of the pulmonary arteries. These factors, alone or in association, lead to insufficiency of the coronary circulation.

Morphologic evidence of coronary insufficiency in cases of embolism of the pulmonary artery is more likely to occur if there are recurrent embolization, narrowing of the coronary arteries, cardiac hypertrophy and adequate duration of life after embolism. Anatomic changes in the myocardium in persons with embolism of the pulmonary artery may be considered the end result of the myocardial ischemia which accounts for the characteristic electrocardiographic changes. The resemblance of electrocardiographic changes in cases of embolism of the pulmonary artery to those in cases of myocardial infarction of the posterior wall may be explained by the diminished flow through the right coronary artery resulting from increased tension in the right ventricle.

FROM AUTHORS' SUMMARY

OCCURRENCE AND DISTRIBUTION OF CALCIFIED PLAQUES IN THE SPINAL ARACHNOID IN MAN E Y HERREN, *Arch Neurol & Psychiat* **41** 1180, 1939

The cells of the spinal arachnoid membrane often form whorls and become calcified, producing so-called plaques. Weed considered the plaques a manifestation of advanced age, with which opinion Herren does not agree. Herren studied the distribution and location of the plaques in human beings of various ages. He found the largest number of plaques in the dorsolumbar and the lumbosacral areas, and especially on the posterior surface of the cord. Persons without plaques were on the average eight and eight-tenths years older than those with plaques. The plaques form by deposition of calcium in the whorls of the arachnoid cells, but why they should arise with preference in certain areas, Herren could not explain.

GEORGE B HASSIN

CHRONIC ULCERATIVE CECITIS IN THE RAT B F JONES and H L STEWART, *Pub Health Rep* **54** 172, 1939

A brief description is presented of a spontaneous disease of rats characterized by chronic ulcerative cecitis and chronic lymphangitis, lymphedema and lymphoid hyperplasia of the lymph nodes of the mesentery.

FROM AUTHORS' SUMMARY

CYCLIC CHANGES IN THE CHROMATIN OF THE NUCLEI OF THE ENDOMETRIUM  
R CIVILAND, Surg, Gynec & Obst **69** 18, 1939

A study of 200 specimens of human endometrium obtained from normal women and from others exhibiting various degrees of ovarian failure revealed that two forms of nuclei can be distinguished, namely, a granular and a nongranular, or solid, homogeneous, form. Further, the granular form of nucleus showed two distinct types of distribution of the chromatin, aggregate and diffuse. The former type of distribution appeared almost uniformly in the nuclei of endometrium presenting the characteristics of proliferation. It was not predominant in the secretory or menstrual phases. It appears, then, that the aggregate type of distribution of the chromatin is characteristic of human endometrium which is under the influence of the follicular hormone alone either normally or as a manifestation of second degree ovarian failure. As a whole, tissue from women with first degree ovarian failure showed the aggregate type of chromatin in the stroma more frequently than did tissue obtained from normal women during the secretory and menstrual periods. On the other hand, the gland chromatin in endometrium associated with first degree ovarian failure showed no significant differences from normal. These observations suggest that differences in the threshold response of the gland and stromal nuclei of the human endometrium to hormonal stimulation may furnish the basis for determining fluctuations in endocrine levels.

FROM THE AUTHOR'S SUMMARY (WARREN C. HUNTER)

HISTOLOGY OF FREI'S REACTION. F. FRANCHI, *Gior ital di dermat e sit* **80** 369, 1939

The Frei intradermal reaction in the presence of venereal lymphogranuloma has a tuberculoid structure in which epithelioid cells and giant cells of the Langhans type are prominent elements. In the presence of venereal lymphogranuloma the reaction to the Frei antigen is specific, the reactions to bacterial vaccines are nonspecific and of simple inflammatory nature.

CHANGES IN THE UMBILICAL ARTERY. G. SCHALLOCK, *Virchows Arch f path Anat* **302** 195, 1938

The umbilical artery was studied histologically during the fetal period and at varying periods after birth. Ligation of the cord after birth leads to immediate contraction of the vessel, with swelling of the subendothelial ground substance. Contraction is followed by dilatation, during which the ground substance increases and histolytic changes occur. The further changes consist of necrosis of the wall with or without thrombosis, arteriosclerosis with or without obliteration of the lumen, hyperplasia especially of the elastic tissue, and proliferative changes with formation of a new lumen. These changes are not due to increased pressure in the artery but are an adaptation to the functional alterations that follow ligation of the cord. The escape of blood or serum into the wall of the vessel is an etiologic factor in the later changes.

O. T. SCHULTZ

INVOLVEMENT OF BONE IN LEUKEMIA. K. APIITZ, *Virchows Arch f path Anat* **302** 301, 1938

Apitz presents a detailed histologic description of the skeletal changes in 2 cases of lymphoid leukemia and describes three types of involvement. The least frequent manifestation of the disease is localized erosion of bone through lacunar resorption. This is looked on as evidence of an invasive or neoplastic character of leukemic cells. Necrosis of the infiltrated marrow may lead to death of bone and to spontaneous fractures, this form of skeletal involvement is not due to aggressive growth of the leukemic tissue. Leukemic infiltration of the marrow may lead to osteoporosis and localized atrophy of bone with spontaneous fracture. In children leukemia may interfere with bone growth and may be associated with subperiosteal bone formation.

O. T. SCHULTZ

## Microbiology and Parasitology

MOOSE ENCEPHALITIS L S KING, *Am J Path* **15** 445, 1939

A subacute or chronic leukoencephalitis occurring naturally in moose is described. The characteristic picture consists of a mild degree of perivascular demyelination, with formation of neutral fat and with fibrous gliosis disproportionate in extent to the loss of myelin. There may be mild inflammation restricted to the white matter. There is evidence suggestive that a primary inflammatory reaction involving gray matter observed in 1 of 8 animals may represent a separate condition. Attempted animal passage of fresh material from an infected moose was unsuccessful. The cause of this leukoencephalitis is obscure, but various possibilities are discussed.

FROM AUTHOR'S SUMMARY

PNEUMONIA PRODUCED BY TYPHUS RICKETTSIAE M R CASTANEDA, *Am J Path* **15** 467, 1939

The intranasal inoculation of mice and rats with typhus virus (orchestic variety) has given rise to hemorrhagic lesions of the lungs which kill the mice in ninety-six hours and the rats in seventy-two hours. The lungs show, in sections and smears, considerable numbers of rickettsia bodies, which have been obtained in pure suspension by grinding and fractional centrifugation. Rabbits have been infected by the intratracheal route with or without forcing down the body temperature. Hemorrhagic pneumonia develops, and rickettsias are present in large numbers in smears and in sections of the lungs, but the animals subjected to a low body temperature present greater quantities of rickettsia bodies. These rabbits die in from forty-eight to ninety-six hours after inoculation. The rabbits not submitted to a low body temperature die after a longer period of time and show lesions of the lungs which are more extensive but which contain fewer rickettsias. To produce massive infection of the lungs it is necessary to inoculate considerable numbers of rickettsias. This method of cultivating rickettsias has proved useful in obtaining typhus vaccine for practical purposes.

FROM AUTHOR'S SUMMARY

DIABETES AND PULMONARY TUBERCULOSIS H F ROOT and W T BLOOR, *Am Rev Tuberc* **39** 714, 1939

A study of the various etiologic factors in the pulmonary tuberculosis of 364 diabetic patients points to the disturbed nutrition of the diabetic patients as next in importance to their contact with patients with open tuberculous lesions. The incidence of pulmonary tuberculosis in adults with diabetes does not show a decrease corresponding with the general decrease in mortality from tuberculosis in the community. Pulmonary tuberculosis followed the onset of diabetes in 83 per cent of the cases. In 126 autopsies on diabetic patients with pulmonary tuberculosis it was found that the outstanding features were many healed foci, a tendency toward the formation of tough, fibrous pleural adhesions and a high frequency of caseating lesions with cavitation. On chemical analysis the lungs of these patients showed strikingly lower concentrations of phospholipid and lipid than the lungs of nondiabetic patients. The advantages of protamine zinc insulin in the treatment of tuberculous diabetic patients are discussed, and dietary information is given. Diabetic patients make excellent subjects for pneumothorax and thoracoplasty. The prognosis for the diabetic patient with pulmonary tuberculosis has been greatly improved by insulin and can be further enhanced by early diagnosis. Routine roentgen examination of every diabetic person's chest is recommended.

H J CORPER

ORAL TUBERCULOSIS J C BRYANT, *Am Rev Tuberc* **39** 738, 1939

Tuberculous involvement of the oral cavity is comparatively rare, only 17 cases have been detected in some 7,000 cases of far advanced tuberculosis over a period of eighteen years. Tuberculous lesions of the tongue frequently have a history of mechanical irritation from the sharp edges of decayed and abraded teeth, broken silver fillings, gold inlays or crowns, or broken artificial teeth. These sharp edges traumatize the tissue. The constant irrigation and bathing of the oral tissues by the salivary and mucous secretions render the oral tissues highly resistant to tuberculous infection. Tuberculosis of the oral cavity is a secondary manifestation of a far advanced pulmonary condition with an unfavorable prognosis. When the prognosis is favorable, tuberculous lesions are seldom formed in tooth sockets following extractions, despite the presence of sputum heavily laden with the bacilli.

H J CORPER

PATHOGENICITY OF AVIRULENT PNEUMOCOCCI FOR ANIMALS DEPRIVED OF LEUKOCYTES A R RICH and C M MCKEE, *Bull Johns Hopkins Hosp* **64** 434, 1939

When nonencapsulated pneumococci, which are nonpathogenic (avirulent) for normal rabbits, were introduced into the tissues of rabbits deprived of leukocytes, they exhibited the qualities of virulent pneumococci, i. e., they multiplied to enormous numbers and produced a progressive local infection, leading in one third of the animals to septicemia. Recovered from the blood at the death of the animal, the organisms were still nonencapsulated and avirulent for rabbits possessing leukocytes. Since in the leukopenic animal the avirulent pneumococcus behaves as a virulent pathogen, the transformation of the virulent, encapsulated form into the avirulent, nonencapsulated form entails no change which prevents its survival and free proliferation in the tissues if it is unmolested by phagocytes. The local lesion produced by the nonencapsulated, avirulent pneumococcus in the leukopenic animal exhibits the hemorrhages and necrosis of tissue familiar in the lesions produced by the encapsulated, virulent pneumococcus in the normal animal. The factor responsible for virulence (the polysaccharide capsule) is therefore not necessary for the production of the characteristic type of tissue damage that virulent pneumococci produce. The power to damage the tissues so resides in the somatic rather than in the capsular portion of the bacterium, and the change in the organism resulting in the failure to produce a capsule does not alter the ability of the somatic portion to produce the characteristic lesion.

FROM AUTHORS' SUMMARY

MENINGOCOCCUS INFECTION OF CHICK EMBRYO G J BUDDINGH and A D POLK, *J Exper Med* **70** 485, 499 and 511, 1939

It has been found that the meningitis in the chick embryo following inoculation of meningococci into the body wall or into the amniotic cavity is due to invasion by way of the blood stream only.

STUDIES ON EASTERN EQUINE ENCEPHALOMYELITIS L S KING, *J Exper Med* **70** 675 and 691, 1939

*Genesis of the Disease in the Guinea Pig*—The virus of Eastern equine encephalomyelitis when injected peripherally into the guinea pig invades the blood stream and passes directly from the blood stream into the brain. This seems to be the principal, though not necessarily the exclusive, route of infection. Once the virus is in the nervous system, its further spread may occasionally be determined by anatomic connections.

*Intraocular Infection with Fixed Virus in the Guinea Pig*—The behavior of a fixed strain of Eastern equine encephalomyelitis virus was studied in guinea pigs after intraocular inoculation. Such inoculation concerns the central and not the

peripheral nervous system. The susceptibility to the intraocular injection lies midway between that to the highly effective intracerebral and that to the ineffective peripheral injection. The virus must act for ten to thirteen hours in order to induce a fatal infection. Removal of the inoculated eyeball before the expiration of this interval almost always prevents fatality, although it may allow immunity to develop. At suitable intervals after injection of the virus into the eye, it may be recovered from successive and appropriate optic centers before it is demonstrable in nonoptic portions. Approximately twenty-four hours are required for the virus to reach a significant concentration in the contralateral geniculate body, thirty-six hours, in the contralateral visual cortex. Significant amounts of virus may be present in the optic chiasm and tract prior to involvement of the higher centers. Virus placed in contact with the retina produces an insignificant, essentially non-specific reaction as compared with that produced at the site of direct intracerebral inoculation. In the retina there is no necrosis of ganglion cells unless there is a complicating intraocular infection. In the cerebral visual centers the first reaction is inflammatory and interstitial, and it may appear in the lateral geniculate body as early as twenty-four hours after injection of the virus. Neuronal necrosis is not the primary result of the action of the virus on the nervous system in these experiments. The distribution of lesions in the brain is in excellent agreement with the method of direct testing for virus content, and is far more accurate than the latter. The virus in its primary distribution through the nervous system follows the nerve pathways of the optic system. This occurs within the central nervous system, where presumably there is first an involvement of the body of the nerve cell and then a spread along the cell process or axon.

FROM AUTHOR'S SUMMARIES

INTER-RELATIONSHIPS BETWEEN AMINO ACIDS IN THE NUTRITION OF *BACILLUS ANTHRACIS*. G. P. GLADSTONE, Brit J Exper Path **20** 189, 1939

Gladstone grows *Bacillus anthracis* in a chemically defined amino acid medium. When certain amino acids are omitted singly, growth ceases, and the omitted amino acid thus appears to be indispensable. However, if in addition another particular amino acid is omitted, growth occurs, showing that the first amino acid omitted is not indispensable. It appears that each amino acid is really inhibitory but that when both are present they are stimulative. These observations may have a bearing on conclusions drawn from nutritional studies in animals.

EFFECT OF FOREIGN TISSUE EXTRACTS ON THE EFFICACY OF INFLUENZA VIRUS VACCINES. D. H. ANDREWS and W. SMITH, Brit J Exper Path **20** 305, 1939

Andrews and Smith have attempted to analyze the factors which determine the efficacy of influenza virus vaccines. The detrimental effect of tissue extracts from foreign animal species, especially when inactivated vaccines are employed, makes one fear difficulties in immunizing man satisfactorily. It is suggested, however, by analogy in work on ferrets, that the practical difficulties may be less if one attempts only to reinforce the basic immunity which most human adults possess against influenza.

CHEMOTHERAPY OF EXPERIMENTAL PNEUMONIA. A. VAISMAN, LEVADITI and D. KRASSNOFF, Ann Inst Pasteur **62** 36, 1939

Vaisman, Levaditi and Krassnoff develop the thesis that sulfanilamide and various related compounds (or derivatives thereof produced by the infecting organisms) act primarily by interfering with an increase in capsular substance, with the result that phagocytosis is facilitated. This makes the therapeutic role of these substances a "natural" cure. The thesis is compatible with the observation that organisms are not always killed and that the beneficial results are due to the reactions of the body cells.

M. S. MARSHALL

## Immunology

PROTECTIVE ANTIBODIES IN THE SERUM OF SYPHILITIC RABBITS T B TURNER,  
J Exper Med **69** 867, 1939

During the course of syphilitic infection in rabbits, specific humoral antibodies develop which can be demonstrated by an appropriate "protection test" The presence of these antibodies is associated with a high degree of acquired immunity to the disease

FROM AUTHOR'S CONCLUSION

CELLULAR REACTIONS TO A DYE PROTEIN F R SABIN, J Exper Med **70** 67, 1939

The use of an antigen which can be seen within cells demonstrates that one may stimulate the phagocytic cells either of the liver and spleen or of the tissues and lymph nodes to produce antibodies The appearance of antibodies in the serum correlates with the time when the dye protein is no longer visible within the cells and with the phenomenon of a partial shedding of their surface films It is thus inferred that the cells of the reticuloendothelial system normally produce globulin and that antibody globulin represents the synthesis of a new kind of protein under the influence of an antigen An antigen is a substance which can specifically modify the synthesis of the cytoplasm of the cells of the reticuloendothelial system

FROM AUTHOR'S CONCLUSIONS

BLOOD GROUPS AND MN-TYPES OF ESKIMOS IN EAST GREENLAND V FABRICIUS-HANSEN, J Immunol **36** 523, 1939

In a study of 569 pure Eskimos in the eastern part of Greenland (Angmagssalik district) the percentile frequencies of blood groups O, A, B and AB were 23.9, 56.2, 11.2 and 8.7, respectively, and the frequencies of blood types M, MN and N were 83.48, 15.64 and 0.88, respectively This is the first study of the distribution of the blood type factors M and N in Eskimos The values of the blood group factors A, B and O differ from those previously reported by showing a lower frequency of O and a higher frequency of B

I DAVIDSOHN

IMMUNE REACTIONS OF VIRUS MYXOMATOSUM R E HOFFSTADT and K S PILCHER, J Infect Dis **65** 103, 1939

Elementary bodies of myxomatosis virus similar to those found in rabbit tissue were found in the chorioallantoic membranes of infected developing chick embryos The soluble antigen associated with myxomatosis virus in tissues of infected rabbits was found in the infected chorioallantoic membranes of chick embryos after prolonged serial passage

FROM AUTHORS' SUMMARY

INCIDENCE OF PROSTATIC CARCINOMA E P GAYNOR, Virchows Arch f path Anat **301** 602, 1938

In 1,000 consecutive, unselected necropsies on men aged 40 years and over, the entire prostate was removed and fixed in 10 per cent solution of formaldehyde The prostates of 40 men aged 25 to 40 years were added to the material The material was worked up by the razor section technique of Terry Each prostate entire was cut into slices 2 to 3 mm thick, these were stained with toluidine blue and examined at magnifications of 20 to 30 diameters The anatomic division of the prostate into five lobes, posterior, middle, two lateral and anterior, was accepted, although the middle and anterior lobes often consisted of only a few glandular structures Carcinoma was detected in 191 prostates, a percental incidence of 18.4 The incidence increased progressively with age, from 10.4 per cent in the sixth decade to 40 per cent in the tenth In 162 prostates the tumor did not involve an entire lobe and was not macroscopically visible, in this group

multiple tumors were observed (203 carcinomas in 162 prostates). The posterior lobe was most frequently involved (60 per cent), the middle lobe, very seldom. The earliest localization was in the peripheral portion of the gland, and the inner fibromuscular capsule was frequently invaded. The outer fibrous capsule was rarely penetrated, and growth outside the gland was rarely observed, although growth into the blood vessels and lymphatics was frequently observed. Benign hypertrophy did not appear to be a factor in the development of carcinoma, the latter was relatively no more frequent in hypertrophied prostates than in those of normal size. Atrophy also did not appear to be a factor. Secondary carcinoma of the prostate was observed only twice.

O. T. SCHULTZ

IMMUNOLOGIC RELATIONSHIP OF SHOPE'S RABBIT FIBROMA VIRUS TO THE VIRUS OF INFECTIOUS MYXOMATOSIS. C. E. VAN ROOIJEN and A. J. RHODES, *Zentralbl. f. Bakt. (Abt. 1)* **142** 149, 1938

Specific complement-fixing antibodies have been demonstrated in the serum of rabbits immunized with myxoma virus. Serum of rabbits immunized against Shope's virus contains complement-fixing antibodies for that agent but not for myxoma virus. After complement-fixing antibodies have disappeared from the serum of rabbits immunized to Shope's virus and the animals have been inoculated with myxoma virus, complement-fixing properties specific for the latter appear. Complement-fixing antibodies for myxoma virus occurring in the serum of immune rabbits are usually present for at least eight weeks. Similar properties for Shope's virus generally disappear before this time. The results of these tests evidence no serologic relationship between the two viruses.

PAUL R. CANNON

## Tumors

EFFECT OF FREEZING IN VITRO ON SOME TRANSPLANTABLE MAMMARY TUMORS AND ON NORMAL RAT SKIN. G. B. MIDER and J. J. MORTON, *Am. J. Cancer* **35** 502, 1939

After exposure to  $-74^{\circ}\text{C}$  in vitro, Walker rat carcinoma 256 and mouse sarcomas 180 and 37 grew on subcutaneous transplantation. The effects of the rate of freezing, the duration of the frozen state (up to twenty-four hours), the number of repeated freezings and thawings, and the physical state of the tumor are discussed. The squamous epithelial and connective tissue cells of normal adult rat skin may grow after a single freezing to  $-74^{\circ}\text{C}$ .

FROM AUTHORS' SUMMARY

PRODUCTION OF LUNG TUMORS IN MICE. M. B. SHIMKIN, *Am. J. Cancer* **35** 538, 1939

Mice weighing about 30 Gm tolerated an intratracheal injection of 0.1 cc of water, saline or serum suspension, the mortality from the procedure was about 35 per cent. Primary pulmonary tumors occurred in over 90 per cent of strain A mice within four months after intratracheal introduction of 0.1 mg of 1,2,5,6-dibenzanthracene or methylcholanthrene dispersed in 0.1 cc of horse serum and cholesterol. The intratracheal route of administration is not as convenient or as efficacious as the intravenous.

FROM AUTHOR'S SUMMARY

VITAMIN E AND EXPERIMENTAL TUMORS. C. CARRUTHERS, *Am. J. Cancer* **35** 546, 1939

Two pure strains of mice, A and C57, have been used in a study of the possible effects of dietary content of vitamin E on the incidence and metastasis of tumors induced by methylcholanthrene. The extent and the frequency of spontaneous mammary tumors in the females of susceptible strain A have also been examined.

Synthetic diets containing the usual constituents—casein, lard, starch, salts—and adequate supplies of the vitamins except E proved adequate for growth even when they contained rancid lard, which destroys even traces of vitamin E. Supplemented with vitamin E concentrate prepared from cottonseed oil, these diets are also adequate for reproduction but not for lactation. The administration of vitamin E concentrate had no significant effect on the carcinogenic action of methylcholanthrene dissolved in lard or spermaceti and injected subcutaneously. When spermaceti was used as a solvent tumors arose on the average, twenty and six-tenths days earlier in strain A mice. Conclusions regarding the effect of nutritional factors on carcinogenesis induced by potent cancer-producing hydrocarbons must be drawn with care, the vigorous action of these carcinogens seems to be independent of the nutritive state of the animal. The etiologic nature of tumors induced by methylcholanthrene has been rendered questionable by the fact that many animals, approximately 30 per cent, acquired epidermoid carcinomas, most other investigators have reported sarcomas. The hydrocarbon produces more extensive ulceration in strain A than in strain C57. In both strains the tumors invaded the musculature, 58 per cent of them when the hydrocarbon was dissolved in spermaceti, 82 per cent of them when it was dissolved in lard. In strain A 41 per cent of the animals had nonmetastatic "lung tumors" when spermaceti was used as solvent of the carcinogen, 45 per cent when lard was employed. Methylcholanthrene thus markedly increases the incidence of these tumors which are hereditary characteristic of strain A. Spontaneous mammary carcinomas arose in strain A females whose vitamin E stores permitted a "first litter" fertility. On the rancid diet the incidence of mammary tumors was much lower. Whether this was due to lack of vitamin E or to some other dietary influence has not yet been determined.

FROM AUTHOR'S SUMMARY

THE STATISTICAL RELATION BETWEEN GOITER AND CANCER J. F. McCLENDON,  
Am J Cancer 35 554, 1939

Although the statistics so far studied may not be regarded as entirely sufficient to establish a relation between goiter and cancer, the evidence is pretty conclusive that thyroid adenoma may predispose to cancer of the thyroid, and the evidence is ample to act as a warning that goiter may increase the cancer rate. Therefore prophylaxis against goiter by administration of iodine to the young and administration of desiccated thyroid to the aged may be a precaution worth taking to prevent an increase in the incidence of cancer.

PAINFUL SUBCUTANEOUS TUBERCLE (TUBERCULUM DOLOROSUM) A. P. STOUT,  
Am J Cancer 36 25, 1939

An investigation of 2,081 superficial tumors of skin and subcutaneous tissues showed that 20, or approximately 1 per cent, were associated with attacks of paroxysmal pain. The types of tumor included not only leiomyoma and glomus tumor but also neurofibroma, fibroma, fibrosarcoma, keloid, dermoid cyst and benign epithelioma in a sebaceous cyst. The tuberculum dolorosum, therefore, is not confined to a single form of tumor but may manifest itself in a variety of morphologic types. No adequate explanation for the occurrence of the attacks of paroxysmal pain could be found.

FROM AUTHOR'S SUMMARY

TRANSMISSIBLE MONOCYTOMA OF THE MOUSE M. R. LEWIS, Am J Cancer  
36 34, 1939

The strain-specific transplantable growth designated as monocytoma no 255, which arose from the implantation of a white spot on the spleen of a mouse which had received an intraperitoneal injection of dibenzanthracene four hundred and eleven days previously, is composed of malignant cells of the monocyte type (the majority of them being permanently altered large epithelioid cells) and is transplantable by means of living cells of the same kind.

FROM AUTHOR'S CONCLUSION



CANCER AND JEWS G WOLFF, *Am J Hyg* (Sect A) **29** 121, 1939

These new statistics from Berlin seem to show that there is little real difference between Jews and non-Jews in the general frequency of death from malignant disease if the higher mortality of the youngest age groups is left out of consideration because it depends on small numbers and is opposed to the somewhat lower mortality of Jews in a majority of the other age groups. On the whole, the standardized mortality rate for the Jewish population is slightly less than that for the whole population. These differences tend to disappear and may well be due to changes of social and occupational structure, since mortality from cancer is clearly associated with some occupational differences and perhaps influenced to a certain degree by social factors, for example, opportunities for early diagnosis and treatment. Here, then, racial factors seem to play no part. On the other hand, there is plainly a difference between the two groups in the localizations of the disease. Whether this difference is a racial distinction or the consequence of a difference in habits of life remains obscure. The recent observations of Handley speak against any racial immunity from carcinoma of the uterus, they require confirmation before they can be fully accepted. In general, it seems improbable that in such a racial mixture as the religious community of the Jews must be from the anthropologic point of view there should be racial differences respecting the pathologic nature of malignant disease. It seems far more probable that in this matter habits and customs, eating and drinking, occupations and social status have an influence, limited but distinct, which has not yet been explained. It is true that cancer is distinct from the epidemic diseases, which show seasonal, secular and geographic variations, and is a reasonably constant cause of mortality, depending principally on age. It may be that a study of heredity will bring about clearer conclusions by tracing the course of events in particular "cancer families"—an important if difficult piece of work. This, however, is a problem totally different from that of racial ideology, which has but little in common with the exact study of inheritance.

FROM AUTHOR'S CONCLUSIONS

PINEALOMA A H BAGGENSTOSS and J G LOVR, *Arch Neurol & Psychiat* **41** 1187, 1939

Of 10 cases of tumor of the pineal body (pinealoma), the authors classified 2 as cases of glioma (spongioblastic pinealoma). In 5 cases the structure of the tumor resembled phases in the development of the normal pineal body. For instance, in 3 of these 5 cases the histologic picture suggested the pineal bodies of infants of about 2 and 9 months. In the remaining, the third, group of cases the authors included cases of so-called pineal ependymoma, which, they state, is differentiated from pure ependymoma by the presence of large cells possessing the characteristic features of pineal parenchymal cells. In short, in some cases a pineal tumor arises from parenchymal cells of the pineal body, and in some, from the neurologic cells.

GEORGE B HASSIN

FAMILIAL MAMMARY TUMORS IN THE RABBIT H S N GREENE, *J Exper Med* **70** 147, 159 and 167, 1939

The clinical histories of two different types of familial mammary cancer in the rabbit are given. As to one type, the first clinical sign of an abnormality of the breast was a sudden intense engorgement, after which the disorder passed through stages of cyst formation and benign neoplasia to cancer with metastasis. In regard to the second type, the neoplasia originated in clinically normal breast tissue, and there was no history of antecedent mammary abnormality.

The pathologic histories of two types of familial mammary cancer in the rabbit are given. One type was distinguished by characteristic antecedent mammary

changes similar to those found in Schimmelbusch's disease in women and by a distinctive papillary structure. The second type originated in normal breast tissue and was characterized histologically by an atypical proliferation of acini.

The clinical and the pathologic course of 25 mammary tumors in rabbits are described. The antecedent history of the breast and the morphologic character of the growths allowed a natural classification into two distinct types, one of which was distinguished by preexisting cystic mastitis and a papillary structure, while the other originated in clinically normal mammary tissue and was characterized by an adenomatous structure. The two types of neoplasia occurred almost exclusively in two family groups, and heredity played a fundamental role both in the occurrence of the tumors and in the determination of the type. Endocrine changes comparable with those found in animals after long-continued administration of estrogenic substances occurred in the tumor-bearing rabbits, and it was inferred that the spontaneous growths represented a natural counterpart of the experimental induction of neoplasia with esthane (theelin).

FROM AUTHOR'S SUMMARIES

MANIPULATION OF GROWTH OF FROG CARCINOMA. B. LUCKE and H. SCHLUMBERGER, *J. Exper. Med.* **70** 257, 1939.

The adenocarcinoma which commonly occurs in the kidney of the leopard frog has been transplanted into the anterior chamber of the eye, where the characteristics of its growth have been studied by direct observation with the slit lamp microscope. Such observations have been amplified by photographs taken at intervals as permanent and objective records of the mode of development and progress of the growths from earliest to advanced stages.

FROM AUTHORS' SUMMARY

A CONSIDERATION OF CERTAIN TYPES OF BENIGN TUMORS OF THE PLACENTA. A. A. MARCHETTI, *Surg., Gynec. & Obst.* **68** 733, 1939.

The comparative rarity of chorioangioma is attested by the 217 instances now on record in the literature. From a study of 8 cases Marchetti differentiates several types on the basis of histologic structure and pattern. The cellular or immature type, the vascular or more mature type and the type accompanied by varying degrees of degenerative changes may intermingle or show all gradations in the same tumor. It is fairly well established that the tumor tissue originates from the chorionic mesenchyme, with the proliferating endothelium and blood vessels playing the leading role, while the stroma has a subordinate part or is passive. It is still an open question whether the placental chorioangioma is a true tumor or a malformation. Chorioangioma is of little clinical significance.

WARREN C. HUNTER

CARCINOGENIC AGENTS PRESENT IN THE ATMOSPHERE AND THE INCIDENCE OF PRIMARY LUNG TUMORS IN MICE. J. A. CAMPBELL, *Brit. J. Exper. Path.* **20** 122, 1939.

Campbell finds that dust from tarred roads and chimney soot contain carcinogenic agents. The former definitely increases the incidence of primary pulmonary tumors in mice, the latter produces little change. The road contains other agents (inorganic substances) which aid the tar in producing its effects. The carbon of the soot mitigates the effects of the tar in chimney soot.

A COMPARISON OF SOME CARCINOGENIC WITH NONCARCINOGENIC COMPOUNDS AS TO PHOTODYNAMIC ACTIVITY. I. DONIAC, *Brit. J. Exper. Path.* **20** 227, 1939.

Colloid suspensions of 3,4-benzpyrene in water sensitize paramecia to light in dilutions up to 1:100,000,000. The sensitivity of the paramecia is markedly increased by contact with the colloid in the dark. The photo-oxidation products

of 3,4-benzpyrene are also photodynamic, but their action is not increased by contact in the dark. The carcinogenic hydrocarbons 1,2-benzanthracene, cholanthrene and methylcholanthrene have the same action on paramecia as 3,4-benzpyrene. The noncarcinogenic sensitizers to light acridine, acriflavine, eosin and quinine sulfate are less potent photodynamically than 3,4-benzpyrene, and their action is not increased by contact in the dark. The photodynamic property is a sensitive means of assaying biologically the aforementioned carcinogenic hydrocarbons.

FROM AUTHORS SUMMARY

FIBROMA OF THE HEART. A. SIMIONIDIS and A. J. LINZBACH, *Virchows Arch f path Anat* **302** 383, 1938

From the literature relating to so-called fibroma of the heart, a term which usually refers to an organized thrombus, the authors select 6 cases that have distinguishing characteristics. To these they add 3 of their own. One of the patients was a newborn infant, the second a child of 15 months and the third a 53 year old man. The tumors of this special group were congenital and solitary, each was situated in the myocardium and increased slowly in size with the growth of the heart. They were not encapsulated. They were composed of fibrous and elastic tissue, the latter increasing progressively with the age of the lesion, a fact which is interpreted as evidence of adaptation to cardiac function. They were neither inflammatory nor neoplastic but were congenital maldevelopments. A lesion of the type described is termed a fibroelastic hamartia. It is a maldevelopment of the interstitial tissue of the myocardium in the same way that the cardiac rhabdomyoma is a maldevelopment of the muscle.

O. T. SCHULTZ

FORMATION OF FIBRILS IN CULTURES OF THE JENSEN RAT SARCOMA. MARIAN ROZYNEK, *Virchows Arch f path Anat* **302** 405, 1938

This is an experimental attempt to answer the question: Do the immature cells of rapidly growing tumors permanently lose their power of differentiation, or is this power merely held in abeyance? When the Jensen rat sarcoma, which in the host is very cellular and shows no formation of fibrils, was grown *in vitro*, especially under conditions that retarded growth, it showed formation of collagen and elastic fibrils. The fibrils were produced by the sarcoma cells, the process being identical with the formation of fibrils in cultures of normal connective tissue. When fibril-forming explants were reimplanted into rats, the usual cellular, nonfibrillated neoplasm developed. The findings indicate that the property of differentiation is not permanently lost by the sarcoma cell.

O. T. SCHULTZ

# Society Transactions

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## PATHOLOGICAL SOCIETY OF PHILADELPHIA

BANTER L. CRAWFORD, *President*

H. L. RATCLIFF, *Secretary*

*Regular Meeting March 9, 1930*

### Laxity as to the Qualifications of an Expert in the Commonwealth of Pennsylvania as Illustrated by Recent Legal Testimony HERBERT LUND

This is a report of legal testimony given by a pharmacist-chemist who for years has practiced certain important branches of hematology and serology. It points out a weakness in our judicial system. A recent murder trial forms the basis of the report (Commonwealth of Pennsylvania vs James A. Reilly and others in the court of Oyer and Terminer of Fayette County, Pa., 11/67, December term, 1936). The pharmacist-chemist testified for the Commonwealth of Pennsylvania as to the nature of certain dry stains. According to his report, he identified the stains as blood and determined the groups. He used a physiologically unsound method in determining the groups of the dried blood, attempting to recover the erythrocytes in saline solution and agglutinate them, a method similar to that usually used in typing fresh blood. Such an application of the method is not advocated by any textbook or authority. I questioned his results and demonstrated a fallacy of the method to the jury. The point was made that unless the dry red cells were fixed and insoluble they would hemolyze when aqueous solutions were added (Guthrie, C. C. *Am J Physiol* 8:441, 1903), and no cells would be left for the agglutination reactions. The pharmacist-chemist when subjected to a coached cross examination displayed a surprising ignorance of elementary hematology and serology. He admitted that he had never examined blood microscopically except under low magnification (100 diameters). He admitted that he had never identified leukocytes "because they are so in the minority, it is a mighty hard thing to find microscopically." He knew of no books that described leukocytes. He did not know that stains could be used to bring out differences in blood cells. He repeatedly stated and affirmed that erythrocytes after months of drying were still alive "in every sense of the word," because if they were dead they would hemolyze and could no longer be seen. He consistently and repeatedly described erythrocytes as having nuclei (in contrast to leukocytes) and as being larger than leukocytes. He did not know of the concave surfaces of the erythrocytes.

Grouping of dried blood stains is one of the most difficult branches of serology, yet the pharmacist-chemist undertook this work with practically no elementary experience. He admitted that the only fresh blood he had ever typed was his own. In spite of this inadequate training he had, on many occasions, testified in the courts of Pennsylvania and nearby states as to the groups of dried blood stains.

This illustrates the shortcomings of a system in which the qualifications of experts are judged by laymen. It is apparent that laymen cannot evaluate an expert's technical knowledge or the adequacy of his methods but must base their decisions on his age, years of experience, college degrees, official titles and personal appearance. Laymen do not appreciate the limitations of an expert's field, and there is a tendency to enlarge his scope. In this case, serology was

considered well within the field of a general chemist. Cross examination under ordinary circumstances is inadequate. In many cases the object of cross examination is to embarrass the witness and make him lose prestige rather than to evaluate his ability. Usually, incorrect answers are not recognized by the judge and jury. When disputed, the witness' "opinion as an expert" makes a simple question appear to be at best a highly controversial point. Even when the opinion is contested by one qualified to do so, the matter simmers down to one man's word against another's.

### The Need for Improvement in Medicolegal Investigation T. A. GONZALES, Chief Medical Examiner, New York

Of the various branches of medical science, forensic medicine has received the least attention. Few universities have provided in their curriculums courses of instruction in this science. The opportunity to acquire experience in the subject by practical application is presented only in the larger cities, where sufficient material may be available. As a result, the lack of trained experts in this field may be responsible in some degree for the inferior quality of the medicolegal investigations in the majority of jurisdictions in this country.

Other factors are the tenacious retention of the coroner system with its many inadequacies and the failure in some communities to recognize that accurate establishment of the causes of death and other medical facts in cases of violent, suspicious or sudden unexpected death is purely a medical function and should not be relegated to lay coroners' juries.

The coroner's being an elected official (often a layman) with a short tenure of office and the combination of medical and magisterial functions are the fundamental weaknesses of the coroner system. Where efforts have been made to divorce the medical from the judicial functions, the necessity for trained medical investigators has become apparent.

The establishment of the medical examiner system in the city of New York in 1918 created a sound basis for future development. In 1927 Essex County (Newark), N. J., adopted it. This system is purely investigatory, and its primary function is to establish accurately the causes of death and other medical facts in cases coming under its jurisdiction. The law which created the New York office of medical examiner requires that the personnel shall be selected from the Classified Civil Service, with a permanent tenure of office. It specifically indicates the methods to be pursued in investigations, particularly in regard to the visit to the scene and the investigation of the circumstances of the death, the jurisdiction of the medical examiner over the body, the form of report and the power of the medical examiner to perform a necropsy when in his opinion a necropsy is necessary. It also empowers him to take possession of any object found at the scene which in his opinion is necessary for the investigation.

Laboratory facilities are provided for toxicologic, chemical, histologic, bacteriologic and serologic studies. While the necropsy remains the essential procedure, there is a certain percentage of cases in which it fails to reveal the cause of death, and it is then that the laboratory investigations become important. The necessity to determine the presence of alcohol and other drugs in cases of death by violence, unexplained death or suicide by poison is well established. For this purpose a toxicologic laboratory is indispensable. The establishment of the blood groups should be a routine procedure in explaining a violent death, and the examination of blood spots found at the scene or on the clothing of the victim or the suspect when a homicide has occurred is also essential in some cases. Postmortem bacteriologic investigations are often the means of clearing up cases in which obscure types of infections are a factor.

To summarize, the modern medicolegal system should be an impartial fact-finding medical organization, administered by trained medicolegal pathologists and laboratory experts. Adequate facilities should be provided for the practical application of appropriate branches of medical and other sciences to the investiga-

tions. The accurate establishment of causes of death and other medical facts which may be useful for presentation in court should be the primary consideration. In such an organization magisterial functions have no place, they should be relegated to the judiciary, where they belong, i. e. to the magistrates' court, the grand jury and the criminal courts.

### The Coroner and the Medical Examiner R. P. CUSTER

"If there is virtue in antiquity, the coroner's office must have it" (Schultz, O. T., and Morgan, E. M. *The Coroner and the Medical Examiner*, with a Supplement on Medical Testimony by E. M. Morgan, Bulletin 64, National Research Council, Committee on Medico-Legal Problems, Washington, D. C., National Research Council, 1928). It is definitely known to have existed in England as far back as 1194, the coroner maintaining an important status as a representative of the crown and a conservator of the peace bringing criminals to justice and acting in default of the sheriff. His most significant duty then, as now, was to hold inquests on the bodies of those supposed to have died by violence, by accident or in prison. His qualifications were knighthood, residence in the county and property.

Transplantation of the office to our system of government was natural enough, the duties, however, were confined largely to the investigation of deaths occurring under unusual circumstances especially those in connection with which violence or criminality was suspected. Briefly, the coroner must decide, after a survey of the case, whether an inquest shall be held, if an inquest is to be held, usually with the aid of a jury and evidence submitted by his deputies and medical assistants, he must decide not only the cause of death but also what person, if anybody, is responsible and must initiate steps for the apprehension and indictment of any one accused. Thus, the coroner's endeavor assumes both the medical and the judicial aspects of criminal investigation, including study of the corpus delict and the hearing of testimony of witnesses. Unfortunately, the coroner in this country was made an elective officer of the county, subject to the vagaries of partisan politics, he is none too well paid and usually is hampered by the fact that the budget is too niggardly for the maintenance of a competent staff of well trained, properly equipped assistants, oft-times he is forced to employ meretricious vote getters. Although his duties are both medical and judicial, he is rarely required to be a physician or a lawyer, and seldom is. In Britain, where the office of coroner is still appointive as recently as 1926 previous inadequacies in this respect were partially corrected by an amendment stating that the incumbent must be a "barrister, solicitor or legally qualified medical practitioner of not less than 5 years' standing in his profession", his tenure of office is permanent with provision for pension. But even with these improvements the English system falls far short of that of the European continent, where institutes of forensic medicine assume major standing in university structure and where crime detection has attained high repute.

As regards the coroner's physician, he is rarely a career pathologist and practically never devotes full time to the work, he is thereby definitely handicapped in the duties expected of him. Nor can any blame be attached to him. It would be utterly ridiculous to ask a physician to give up his established practice for an inadequately salaried office, the tenure of which depends on the uncontrolled will of the coroner and changes in party administration. The coroner's physician rarely, if ever, is an eye witness to the actual scene of death with body and environs undisturbed. Consequently he must sometimes deal with what is essentially a medical problem on data furnished by the police or a deputy coroner.

In the main, facilities for a complete medical survey of a case are woefully lacking. The equipment for postmortem examinations is usually mediocre, not uncommonly of the worst. How often is the coroner's physician handicapped, even at an impasse, in the final intelligent interpretation of his gross findings through lack of microscopic examinations of tissues and bacteriologic and chemical studies?

In sharp contrast to all of this, Dr Gonzales has given an impressive picture of a well organized, effectively functioning medical examiner's office, still undermanned, however, and with too limited a budget to care completely for the tremendous duties imposed by a metropolitan population. Again, the system affects only the city of New York, rather than being statewide as it is in Massachusetts. A weakness in the system in Massachusetts lies in the fact that outside of Suffolk County (Boston) the examiners are largely physicians, not pathologists, so that at least some of the work and records lack uniformity and completeness.

The obvious advantages of such a system have been demonstrated, and the analysis by Schultz and Morgan, from which much of my material has been drawn, offers an utter condemnation of the old and outmoded coroner's office. It is apparent that the adoption of the medical examiner system would (a) withdraw the office from politics, (b) unify and vastly improve the investigation of death occurring under unusual circumstances, in close harmony with police and legal offices, (c) force improvement in laboratory facilities in rural districts and maintain a check on the standards of autopsy studies in all hospitals, (d) augment the teaching of forensic medicine in medical and law schools, and (e) encourage research and publication in forensic medicine.

It should be emphasized finally that salaries must be adequate and that graded increases in salary and rank must be established to furnish incentive for men of high caliber to accept the responsible positions and for young men of equal quality to enter this branch of medicine as a career. Even so, the ultimate cost to state and county would show little if any increase, more probably a decrease.

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## NEW ENGLAND PATHOLOGICAL SOCIETY

SIDNEY FARBER, *President*

BENJAMIN CASTLEMAN, *Secretary*

*Regular Monthly Meeting, Oct 19, 1939*

### Effect of Irradiation on the Blood CHARLES E DUNLAP

The circulating blood cells are quite resistant to destruction *in vitro* by radiation. It is probable therefore that changes in the peripheral blood picture depend in large part on damage to blood-forming organs. The major damage affects tissues lying within the field of irradiation, but lesser degrees of injury occur in distant bone marrow and lymphoid organs.

The lymphocytes, polymorphonuclear leukocytes and red cells do not show parallel responses to any one technic of irradiation. The resulting blood picture represents a balance between the vulnerability of each cell type and its powers of recovery. Lymphoid organs are highly radiosensitive. They show early and marked damage after small doses but they also recover rapidly from injury. Granulopoietic foci in the bone marrow are moderately radiosensitive, but they recover a little more slowly and less completely than lymphoid organs. Erythropoietic foci are fairly radioresistant, but, once injured, they show poor powers of recovery.

After a single therapeutic exposure to a fairly heavy dose of radiation, a decrease in the number of circulating lymphocytes can be detected within an hour. The decrease continues for about three days and is followed by recovery to normal or above in thirty to sixty days. The polymorphonuclear leukocytes show a transient increase in number, reaching a maximum about twelve hours after exposure. This probably represents mobilization rather than new formation of cells. Subsequently the polymorphonuclear cells fall below normal, reaching a

minimum value some six days after exposure and then recovering to normal a little more slowly than the lymphocytes. The red cells are seldom affected by a single therapeutic exposure but may show a slight decrease in number beginning a week or ten days after exposure. During the first week or two after treatment a few degenerating white cells may be found in circulation. There is a slight shift to the left in the Ainch count, and in some cases mild reticulocytosis occurs. The behavior of the basophils, eosinophils and monocytes has not been adequately studied, but they probably follow the neutrophilic granulocytes. The blood platelets probably increase after small doses and certainly decrease after massive doses.

Patients seldom show serious damage of blood by radiation unless subjected to treatment with massive doses or to prolonged treatment. Radiologists and their technicians, through slight carelessness, may expose themselves to repeated small doses of radiation and suffer significant injury of the blood. Massive overexposure damages cells of all types and leads to death from agranulocytosis or anemia. Slight or moderate chronic overexposure results in leukopenia with relative or absolute lymphocytosis, often associated with anemia. Radiation anemias are ordinarily characterized by parallel reductions of red cells and hemoglobin without change in the appearance of the remaining blood cells. In a considerable number of cases, however, one notes an elevated color index with anisocytosis and poikilocytosis, an occasional slight increase in blood bilirubin and rare megalyocytes. Associated with leukopenia and relative lymphocytosis, this picture may be difficult to distinguish from pernicious anemia.

Occasionally radiologic workers show bizarre regenerative states, probably resulting from overcompensation of the blood-forming organs for repeated slight injury. Various degrees of lymphocytosis, monocytosis, eosinophilia and erythrocytosis have been described. Since 1911 reports of 28 instances of leukemia occurring in radiologic workers have appeared. Both lymphatic and myelogenous leukemia have followed exposure to roentgen rays as well as that to radioactive compounds. Some doubt exists as to the etiologic role of radiation in these cases even though several workers have shown that the incidence of spontaneous leukemia in experimental animals can be greatly increased by repeated small doses of radiation.

The treatment for abnormalities of the blood resulting from irradiation is immediate removal of the subject from further exposure. Recovery under these conditions is usually uneventful. Radiologic workers should have periodic complete examinations of their blood. Leukopenia with relative lymphocytosis is evidence of inadequate protection, and anemia indicates that severe damage of the blood has already taken place.

#### DISCUSSION

SHIELDS WARREN. There is little that I can add to the summary that Dr Dunlap has given so clearly, but there are a few points that I should like to stress a little more than he has. In the first place, it is generally the responsibility of the pathologist to keep an eye on the hematologic work of the hospital, and it is consequently the responsibility of the pathologist to see that the hospital radiologists and technicians do not get into difficulty. I am afraid pathologists do not accept that responsibility as seriously as they should. While the amount of 0.2 roentgen has been stated as a probably safe amount, it has been stated arbitrarily. There is no exact knowledge on the subject, and, moreover, it is hard for one working with roentgen rays or radium to get average exposure. Consequently I feel that the pathologist will be doing a real service to his medical colleagues in the fields of roentgen radiation and radium if he keeps a careful eye on their blood and notes any of these changes that may take place. As Dr Dunlap has pointed out, recovery is usual, and given half a chance, say six weeks out in the sun, it is amazing how completely the normal blood picture may come back.

KENNETH LIVINGSTONE. In order to gain an impression of the changes in the white cell picture following the application of roentgen rays of high voltage



over large fields, we have reviewed 12 cases in which treatment of this type was used during the past six months at the Collis P Huntington Hospital. The million volt machine was used, the patients receiving from 800 to 9,000 roentgens over a 30 by 30 cm field. No patient showed any indication that anemia was developing during treatment. All showed definite leukopenia. This reduction affected all the white cell forms except in 2 patients who had malignant lymphoma with very low lymphocyte counts before treatment, these 2 patients showed no change in the lymphocytes. Compared on the basis of relative change in total number of cells, the lymphocytes were the most labile of the white cell elements, the mononuclears showed moderate sensitivity, and the polymorphonuclear series was most resistant during the period of treatment. In several cases in which the blood was studied three to six weeks after completion of the course of treatment, it gave evidence of continued depression of the polymorphonuclear series although the lymphocytes and mononuclears were apparently in the rebound phase described by Dr Dunlap. In 8 of the cases young forms of the polymorphonuclear series either appeared or increased in number during treatment. The impressions gained from these few cases are that the lymphocytes are the most labile of the white cells—their response to injury is quantitatively marked—but that their recovery is rapid, that the polymorphonuclear series is relatively resistant to injury but that the effect of injury is more prolonged, and that the mononuclear series is intermediate in reaction and less consistent than the other two forms.

TRACY B MALORY. Since Dr Warren has raised the question in regard to radiologists, I think I might mention the slight experience we have had at the Massachusetts General Hospital. During the last two years my associates and I have been examining the staff at frequent intervals. I think it is probable that the figures just heard here are in general those for persons who have had one or more heavy treatments, and it is quite likely that the picture resulting from chronic minimal exposure may turn out different from that in the x-ray department. At the Massachusetts General Hospital a considerable percentage of the doctors and technicians have white cell counts running distinctly above normal limits, as high as 15,000 in some instances. One patient has a red cell count of 6,000,000. In other words, the element here seems to be that of stimulation rather than depression, and, since the parallelism of benzene poisoning was brought out it is also worth remembering that in a considerable percentage of cases of mild exposure to benzene increase rather than decrease in both white and red cell counts was noted.

GEORGE WATT. What could be considered a good interval, or how many examinations should a person exposed to roentgen rays have, to be adequately protected?

SHIELDS WARREN. Examination about once a month should enable one to detect any early change in the blood.

WILLIAM FREEMAN. Given a patient who has moderate anemia established from roentgen radiation, is any specific therapy effective?

CHARLES E DUNLAP. I do not believe there is any specific treatment for the anemia. One merely stops the exposure to radiation until recovery has proved adequate.

SIDNEY FARBER. Has there been any correlation between age and radiation effect?

CHARLES E DUNLAP. A few experiments show that younger animals are a great deal more sensitive than older animals. A pregnant rabbit irradiated two days before delivery and two days after delivery will die of anemia. Young rats irradiated shortly after birth will have more profound anemia in a shorter time than older rabbits. I do not know of any work on human beings.

**Fat Embolism** LORNE M. GRAY

Fat embolism was produced in rabbits by intravenous injections of fat extracted from the long bones of rabbits. The fatal dose was found to be between 0.8 cc and 1.1 cc per kilogram of body weight. One series of rabbits was given increasing doses of fat (0.1 to 0.8 cc per kilogram), the animals were killed in twenty-four hours, and the total fat was extracted from the lungs. Another series was given sublethal doses of 0.5 cc per kilogram, the rabbits were killed in one to seven days, and the total fat was extracted from the lungs. This formed a basis for the study of the distribution of fat in the body and its disappearance from the lungs. This was compared with the histologic picture in the lungs.

Various emulsifying agents were used, but these failed to effect recovery in rabbits given injections of fat.

One series of rabbits, however, when started on small doses and given gradually increasing doses over a period of three to four weeks became tolerant to fat, so that they showed no ill effects on receiving in single successive doses amounts which ordinarily would cause their death. This tolerance to fat has been reported by two other groups of investigators but remains unexplained.

The correlation of many experimental results to observations in patients with fatal fat embolism is conflicting. The histologic picture is very similar, but the fatal human dose and the certain origin of this embolic fat are still under dispute.

## DISCUSSION

**BENJAMIN CASTLEMAN** Were histologic sections of the lungs made and, if so, was there anything that suggested lipoid pneumonia?

**LORNE M. GRAY** The question of lipoid pneumonia is an interesting one. The usual reaction to fat embolism in the rabbit is a few polymorphonuclears and congestion of blood vessels in the lung—hardly comparable to human lipoid pneumonia, in which mononuclear fat-laden phagocytes predominate. However, in animals that have lived a month after the last injection there is some fat in the alveoli. In some of the human beings whose cases are reported in the literature large areas of hemorrhage were present around the fat, but I have never seen that to any extent in experimental animals.

**DONALD A. NICKERSON** Is there any correlation between the amount of tissue in the lungs containing fat emboli and fatality?

**LORNE M. GRAY** The distribution of fat in the lungs is more or less patchy in the experimental animal and in man. Fat is present in the capillaries, arterioles and alveolar spaces, but the longer those animals which have received sublethal doses live before they are killed the less the fat that is seen in capillaries. The spread appears to be from capillaries to arterioles and then into alveolar spaces.

**Relation of Chronic Mastitis to Carcinoma** SHIELDS WARREN

Although chronic mastitis has been recognized as an entity, and although its relationship to carcinoma of the breast has been under discussion for over a hundred years, there have not been made available as yet any generally accepted data that would permit the surgeon to know what chance of development of carcinoma he requires his patient to take when he leaves a focus of chronic mastitis in the breast. Most studies have been carried out on the basis of finding evidence of coexisting carcinoma and mastitis in the surgically removed material. Two difficulties are encountered with this method of investigation. First, any appreciable degree of association might be coincidence. Second, the mastitic changes might be the result rather than the precursor of the carcinoma.

Since the practical question that the pathologist and the surgeon have to face is what will happen to the breast showing chronic mastitis, it seemed that the best way of dealing with the subject was to follow an appreciable group of cases for a long enough period of time to gain a fair idea as to what the ultimate behavior of such a breast might be.

The present study was undertaken from that standpoint. It is based on 1,206 cases of various types of diseases of the breast which were followed for five years or longer. The exceptions to this follow-up period were the cases in which carcinoma developed in less than five years after the first operation for a benign lesion of the breast.

In order to test further the statistical validity of the results and to insure an adequate variety of the samples selected, 602 cases were taken from the files of the Toronto General Hospital in Ontario and 604 from those of various Boston hospitals. The Boston group was further divided into two samples. These three groups checked with one another closely. The vast majority of the cases were cases of chronic mastitis and chronic cystic mastitis, but there were included 21 cases of adenoma and 70 cases of adenocystoma. Among the latter, there were 7 cases of carcinoma, an incidence of 8 per cent.

In the group of chronic mastitis and chronic cystic mastitis there were 35 cases (a percentage of 3.4) in which cancer developed either in the previously involved breast or in the opposite breast. When the incidence is calculated on the basis of the number of years of exposure and contrasted with the attack rate rather than the mortality rate, the occurrence of cancer is found to be 0.37 per hundred as against a calculated rate of 0.03 per hundred in the entire female population of Massachusetts in 1930.

The average duration of the follow-up study was nine years, and the age distribution of the cancers was such that approximately one half the patients were under 50 and one half over 50 years of age.

One of the most difficult problems is that of determining what the morbidity rate of cancer of the breast in the female population is. I have assumed that the annual mortality rate multiplied by 2 would equal the annual attack rate on the basis that the number of patients cured of cancer of the breast and the number of patients whose cancer was never diagnosed would perhaps equal the number of patients dying from the disease. This is probably an excessively high allowance, but it seems wise to work cautiously in this field and overestimate rather than underestimate the morbidity rate of cancer in the general population.

When one calculates from our material the age-specific cancer rates and contrasts them with those of the Massachusetts population and of Canada, two things stand out in startling fashion. The first is the marked predominance of cancer in the group with antecedent pathologic changes in the breast. The second is that there is nearly 12 times as much cancer of the breast developing in women from 30 to 49 years of age in the previously diseased group as in the general population and only 2.5 times as much as in those over 50 years of age. In other words, the fact of antecedent pathologic changes in the breast has most weight when it concerns a woman in the earlier of these age groups.

When one turns to the histologic observations in those cases in which mastitis was followed by carcinoma, the results are most discouraging. There is no form of lesion singled out, but instead one finds practically any type of lesion being followed by cancer of the breast. In general, intraductal proliferation of the epithelium of the ducts appears to be the most important suggestion of subsequent malignancy. The large columnar acidophilic cells that are occasionally seen lining cysts or projecting in papillary formation seem to be relatively rarely associated with the development of cancer. It is not unnatural that there should be a relatively high rate for the development of cancer from true intraductal papillomas since at times it is possible to see infiltrating carcinoma spreading from the base of the lesion in ordinary histologic material. However, no form of abnormal change in the epithelial elements of the breast seems to be safe. It is my impression that the development of chronic mastitis, chronic cystic mastitis, adenoma or cystadenoma is an expression of an undue ability of the mammary epithelium which predisposes to the development of carcinoma and that no one type of lesion can be regarded as definitely precancerous, on the one hand, or definitely dissociated from subsequent development of carcinoma, on the other hand.

From the practical standpoint, one is faced with the fact that in an average follow-up period of ten years there were 42 cases of cancer of the breast against a calculated expectancy—even conservative adjustment being used—of 13 cases. This incidence of mammary cancer following a preexisting lesion of the breast is not sufficiently great to demand bilateral mastectomy as a preventive measure. The incidence is, however, sufficiently great, particularly among women of the younger age groups, to move one to consider them as constituting a special risk group that should be followed with great care, probably at least at six month intervals, with resort to amputation when any suspicious change in the breast is noted. Lest this last suggestion seem too radical, I add that if one combines the number of patients who have had cancer develop with the number of patients who have had recurrences of their benign mammary lesions necessitating operation, the percentage is strikingly high.

## DISCUSSION

CHANNING C. SIMMONS I have been much interested in hearing Dr. Warren's paper and comparing it with work that Dr. R. B. Greenough and I did several years ago. In 1914 we were struck with the number of cases of cystic mastitis in which malignant disease developed later. We studied a series of 83 cases of cystic disease of the breast in which partial removal was performed. In 4, or 4.8 per cent, cancer developed in the breast which had been partially removed, in 8 per cent further cystic disease developed requiring amputation, in 5 per cent cystic disease developed in the other breast requiring amputation of that breast as well as of the first breast whereas 66 per cent were what might be called "successful cases." Because of the incidence of cancer (4.8 per cent) I now advise simple amputation for cystic disease rather than partial resection. Occasionally I am inveigled into doing partial removal and often I am sorry afterward. As regards the second breast, I have no figures, but in many instances I have had to remove it because of pathologic developments.

IRA T. NATHANSON Three or four years ago Dr. Claude Welch and I were interested in the age incidence of carcinoma of the breast. The figures accepted up to that time were those of Dr. Pack of the Memorial Hospital, New York, which are rather contrary to the general belief, probably because he has a distinctly special group of patients, referred to the hospital only for the treatment of cancer. If one compares the incidence of cancer of the breast in a general hospital with that in the general population I think one is able to show that the incidence of cancer in a general hospital rises steadily with age. Accordingly, we looked up the death certificates in Massachusetts from 1928 to 1930 and, correcting for the fact that one third of all patients with cancer never reached the hospital before death, found a distinct rise in the incidence of carcinoma with age. Therefore these figures confirm Dr. Warren's figures based on a logarithmic curve. The group with mastoplasia present a picture very different clinically in our minds from that observed in the group with frank cystic disease. In questioning a good many patients with carcinoma of the breast as to whether they had cyclic activity in that breast we were struck by the absence of this syndrome.

B. EARLE CLARK One other question I wish Dr. Warren could answer is: What is the incidence of chronic cystic mastitis in the general population? He has pointed out that in a number of cases carcinoma remains undiagnosed, and I think it is fair to assume that there are a number of cases in which chronic cystic mastitis remains undiagnosed, probably much larger than that in which carcinoma remains undiagnosed. Some writers put it as high as 100 per cent, without much specific scientific evidence.

SHIELDS WARREN I have no way of knowing the incidence of chronic cystic mastitis in the general population. On the basis of female hospital population and from what I have been able to gather from the results of routine physical examinations made by various men, cystic disease is not by any means infrequent. On the other hand I should think that 100 per cent is a long way from the truth. There are many women with cystic disease who do not have carcinoma. I think

all physicians can do is to work on the basis of the cases they have been able to study and decide what to do for the patients in the light of present knowledge. If in the course of a routine physical examination cystic mastitis is encountered, it should be regarded as important.

B EARLE CLARK As regards making a separate group of the cases of chronic cystic mastitis, my opinion is that one can frequently find all the histologic features of both groups within the same breast and that a small portion of breast removed for pathologic examination is not a true sample of the whole breast. There may be epithelial hyperplasia in one part and fibrosis in another.

SHIELDS WARREN I am in complete accord with this point of view. Although I first separated the types of breast changes in this study, my results were about the same, so I lumped them all together as one. In the study of chronic mastitis and chronic cystic mastitis there is a tendency to find all gradations.

ROBERT FIENBERG There is one point I should like to have Dr. Warren clear up in relation to the high percentage of carcinoma following cystic mastitis in the lower age groups, which I attribute to hormonal stimulation of the breast. In the general curve, however, showing an increase in the incidence of carcinoma of the breast in older age groups, I am struck by the fact that, especially in the very old, in whom there is a very high incidence of cancer of the breast, there has been no hormonal stimulation for a long time, up to thirty-five to forty years. This long period is inconsistent if the cancer is due to hormonal stimulation. In other words, it seems as if the mechanization of cancer is different in the younger groups with cystic mastitis than in the older groups.

SHIELDS WARREN I should hesitate to claim that chronic mastitis and chronic cystic mastitis are important factors in carcinoma of the breast. All physicians can do on the basis of the data they now have is to deal with these lesions of the breast in the light of those with which they have come in contact. The important thing is to point out that the incidence of carcinoma in any organ, not only in the breast but in the skin, the prostate and the stomach, tends to increase markedly with increasing age. Now a slight hint as to the explanation of this has been thrown out by some of the hormonal work, which suggests that atrophic epithelium is much more likely to respond abnormally with stimulation than epithelium which has not atrophied. Whether that is going to be borne out in the future I cannot say. I do not want you to carry away the idea that any appreciable volume of carcinoma of the breast comes from chronic cystic mastitis. All I am prepared to say is that women with chronic cystic mastitis and those with papillary cyst adenoma represent a group of women that have a special hazard and so deserve particularly careful attention.

## Book Reviews

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**The General Tissue and Humoral Response to an Avirulent Tubercle Bacillus Including Growth Characteristics of the Organism** Sol Roy Rosenthal, M D, Ph D, Associate in Bacteriology and Public Health Joint Contribution from the Tice Laboratories of the City of Chicago Municipal Tuberculosis Sanitarium and the College of Medicine of the University of Illinois Illinois Medical and Dental Monographs, Vol II, No 2 Paper Pp 184, with 80 illustrations Price \$2.50 Urbana University of Illinois Press, 1938

The family of acid-fast bacilli still offers many questions to the investigator. This book represents the efforts of its author to elucidate some of the unknown factors of one member of this family, namely BCG. The author has studied the BCG micro-organism from numerous standpoints and has embellished his record with meticulous detail. The study embraces the effect of fats when incorporated into mediums on bacillary growth. It takes into account the response of the several tissues and organs of the guinea pig's body to infection by various accepted routes, namely, intravenous, intradermal and oral. It records the tissue reaction to injection of fractions of the tubercle bacillus and discusses the so-called submicroscopic forms and their effect on the reticuloendothelial system. It indicates that the life cycle is much like that which Kahn has described for the tubercle bacillus.

The book represents a prodigious amount of work. This in places seems of high order, in other places it gives the impression of lack of discrimination, lack of discretion in listing detail and lack of *critique*. In some areas the detail appears to be laboratory notes almost verbatim. The author presents a great deal of factual information as to the presence of acid-fast bacilli in various organs of the body remote from the site of inoculation, as well as much factual matter on the histologic response in these areas. He appears, at the same time, to have no difficulty in identifying readily all types of cells and cellular structure involved in the areas of reaction. The microscopic studies have been carried out in great detail in practically all the organs of the body and at many periods of time after inoculation, from a few minutes to longer than a year. Blood responses, especially the monocyte-lymphocyte ratio, have received considerable attention.

The contention that the inoculation of bacilli produces prompt effects in remote parts of the body through its influence on the entire reticuloendothelial system is engaging in some respects. It is stated that the cellular response is prompt in remote parts of the body—presumably the reaction is to "submicroscopic" forms of the tubercle bacillus. Organs showing the reaction are culture negative for the most part, but transfer through a series of animals produces sensitiveness to tuberculin and tuberculous disease such as is claimed for the filtrable forms of the bacillus. The submicroscopic forms appear by inference to be bacillary granules or something near filtrable forms. The case for transfer of infectious matter through animals by several passages is suggestive but not convincing.

The book contains numerous, and in places confusing, lapses in sentence structure and shows poor proof reading. Each chapter carries a bibliography.

One is glad to have this book in one's library for reference, yet one cannot but feel that elimination of a great deal of unrelated factual matter might have made the book more attractive and more valuable.

**La ponction sternale. Procédé de diagnostic cytologique** P. Emile-Weil, Médecin des Hôpitaux de Paris, and Suzanne Perles, Chef de laboratoire à l'Hôpital Tanton. Paper. Pp 183, with 25 illustrations and 9 colored charts. Price 75 francs. Paris. Masson & Cie, 1938.

This book reviews the field of application of sternal puncture, pointing out its advantages but also sources of error. The authors in 1936 published a book on

splenic puncture, and Emile Weil is also the author of a volume on diseases of the blood. The present work is the outcome of years of study of material obtained by puncture of the hemopoietic centers (spleen, liver and sternum). The material is divided in two main parts. The first deals with the technic of the puncture, preparation of smears and staining. The cytology of the bone marrow is presented briefly but clearly with the help of some excellent colored drawings of marrow cells. The proportions of the cells in the normal marrow are tabulated in the form of a so-called normal myelogram. The relations of the different cells to one another are then expressed in the form of indexes. The second part deals with the findings in disease. The different leukemias, the leukemoid conditions, the neoplastic primary and metastatic growths of the marrow, the anemias, the polycythemias, the infectious diseases which influence the marrow, some diseases of the liver and spleen (kala azar, malaria, Gaucher's disease and finally Hodgkin's lymphogranuloma and infectious mononucleosis are discussed.

For every disease which presents characteristic changes in the marrow, the cytologic data are tabulated in the form of a myelogram. The differences in the appearance of the cells in the same stage of differentiation in the marrow and in the circulating blood are emphasized, particularly with regard to plasma cells. The concept of the myelogenous origin of the last cell and the implications with regard to the genesis of the so-called plasma cell myeloma are interesting. The sternal puncture is, according to the authors, indispensable for the proper diagnosis and evaluation of the anemias, there, as well as in other conditions, it is essential for the prognosis, for the follow-up and for the control of therapy and as an early indicator of relapses. A good case is made in favor of the study of tissue obtained by puncture of the spleen and occasionally of the liver, in addition to that obtained by sternal puncture. In these relatively early years of sternal puncture the present monograph will serve a good purpose. It radiates an enthusiasm which cannot fail to be transmitted to the reader. It might have been better still if the shortcomings of the method had been stressed a little more adequately. They are known to those who have practiced it critically but are sometimes not sufficiently appreciated by others. Such reserves as to what may be expected would save considerable disappointment. The book can be warmly recommended to all interested in hematology. It has 158 references.

**La mort des brûlés. Étude expérimentale.** L. Christophe. Foreword by L. Binet. Paper. Pages 93, with 20 illustrations and 20 tables. Price 40 francs. Paris: Masson & Cie, 1939.

Following a brief introduction comes a review of the literature on the causes of death resulting from burns. Five chapters deal with the clinical picture and death as due to (1) loss of function of the skin, (2) circulatory disturbances, (3) nervous shock, (4) circulatory shock and (5) intoxications. The author then presents the results of his own investigations, in which he was concerned with death occurring several days after a burn and not with death occurring within a shorter period. The work was done on dogs. Some were burned by means of a Bunsen burner and some by scalding with hot water. Some dogs were not burned but were perfused through the burned limbs of other dogs. Long columns of chemical changes in the blood are recorded. These investigations included also determinations of hemoglobin, of blood and plasma volume and of the sedimentation rate. The results indicate that during the first hours after a burn toxic substances are formed. These circulate in the blood and damage certain histologically demonstrable centers in the anterior part of the hypothalamus, which in turn leads to nephritis characterized by lowering of the chlorides and by elevation of the nonprotein nitrogenous substances. The typical changes in the blood and the occasional gastroduodenal ulcerations are due to the same central lesion. A 7 page bibliography is appended. It is obviously incomplete. Several names are misspelled. The monograph is of considerable practical significance. The therapy must be centered on the first few hours following the burn and should aim at a fixation of the toxic substances in the skin. The lowering of the

chlorides of the blood suggests the importance of the introduction of sodium chloride. The anatomic changes in the central nervous system and in the kidneys are illustrated by 16 photomicrographs.

**Brucellosis in Man and Animals** I Forest Huddleson, D V M, M S, Ph D, Research Professor in Bacteriology, Michigan State College. Contributor Authors A V Hardy, M S, M D, Dr P H, Associate Professor of Epidemiology, DeLamar Institute of Public Health, Columbia University Medical School, Consultant, U S Public Health Service. J E Debono, M D, M R, C P, Professor of Pharmacology and Therapeutics, Royal University of Malta. Ward Giltner, D V M, M S, Dr P H, Dean of Veterinary Division and Professor of Bacteriology, Michigan State College. Cloth. Pp 339, with 40 figures. Price \$3.50. New York: The Commonwealth Fund, 1939.

This book is a revised and expanded edition of "Brucella Infections in Animals and Man" by the same author, published in 1934. The latter was devoted primarily to a description of laboratory methods for the study of brucellosis. The object of the new book is to meet the needs for a more comprehensive presentation of the growing knowledge of the disease, which Charles Nicolle regarded as "a disease of the future." The scope of the book, which is clearly written and nicely printed, is best indicated by an outline of the contents. The three first chapters deal with *Brucella*—the general characteristics of the genus, the methods for its isolation and the differentiation of the species. Then comes the section on brucellosis in human beings, in four parts: an historical survey, brucellosis in the United States (by A V Hardy), brucellosis in Malta (by J E Debono) and treatment. The epidemiologic aspects, the clinical manifestations, the diagnosis and the treatment of the disease receive adequate and competent consideration. In the next chapter brucellosis in animals is reviewed as it occurs in cattle, swine, goats, sheep, other mammals and fowls as well as experimentally in guinea pigs. The various laboratory methods for the diagnosis of brucellosis are well described. In the last chapter Ward Giltner discusses the eradication or control of the sources of brucella infection, which "is a problem in animal hygiene and veterinary medicine, and this is fortunate since veterinary medicine is better organized for the control of this disease than human medicine." There is an appendix of illustrative case reports, a select bibliography of 378 items and a good subject and author index. The book will be of value to physicians, veterinarians and laboratory workers. It is the creditable outcome of individual and cooperative study of the problems of brucellosis.

**Protozoology** Richard R Kudo, D Sc, Associate Professor of Zoology, University of Illinois. Second edition. Cloth. Price \$6.50. Pp 676, with 291 illustrations. Springfield, Ill., and Baltimore, Md., Charles C Thomas, Publisher, 1939.

This volume is the second edition of the author's "Handbook of Protozoology." The change of title was made because of the changes and additions to the text, but the purpose remains an attempt to present "introductory information on the common and representative genera of all groups of both free-living and parasitic Protozoa" to advanced college and graduate students of zoology. The introductory chapter deals with the relationship of protozoology to other fields of biology and gives a short history of the subject. Five chapters are concise statements of the general subjects of ecology, morphology, physiology, reproduction and genetics. Most of the book is devoted to the remaining 37 chapters, which form a taxonomic review of the Phylum Protozoa, in which systematics, morphologic aspects and methods of reproduction are considered and developed in detail. Throughout, the book is well written and beautifully illustrated with pen and ink drawings. Although it is designed as a text for beginners, the taxonomic portion is complete enough to serve as a work of reference for protozoologists who are not specialists in the field. No criticism can be given of the description of the parasitic protozoa, but parasitologists and physicians will be disappointed with the summary treatment of parasitism, immunization and related topics.



## Books Received

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DIVERTICULA AND DIVERTICULITIS OF THE INTESTINE THEIR PATHOLOGY, DIAGNOSIS AND TREATMENT Harold C Edwards, M S, F R C S (England), Surgeon and Lecturer in Surgery to King's College Hospital, London, Surgeon to the Evelina Hospital for Sick Children, London, Jacksonian Essayist 1932, and late Hunterian Professor, Royal College of Surgeons of England Foreword by Gordon Gordon-Taylor, O B E, M S, F R C S Cloth Pp 335, with 223 illustrations Price \$8 Baltimore Williams & Wilkins Company, 1939

VIRUS AND RICKETTSIAL DISEASES WITH ESPECIAL CONSIDERATION OF THEIR PUBLIC HEALTH SIGNIFICANCE A SYMPOSIUM HELD AT THE HARVARD SCHOOL OF PUBLIC HEALTH, JUNE 12-JUNE 17, 1939 Cloth Pp 907, illustrated Price \$6 50 Cambridge, Mass Harvard University Press, 1940

A TEXTBOOK OF LABORATORY DIAGNOSIS WITH CLINICAL APPLICATIONS FOR PRACTITIONERS AND STUDENTS Edwin E Osgood, M D, Associate Professor of Medicine and Head of the Division of Experimental Medicine University of Oregon Medical School Third edition Pp 653, with 37 illustrations Price \$6 Philadelphia P Blakiston's Son & Co, 1940

ANNUAL REPORT OF THE SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE OF THE UNITED STATES FOR THE FISCAL YEAR 1939 Cloth Pp 185 Price 75 cents Washington, D C Superintendent of Documents, Washington, D C, 1939

DISEASES OF THE GALLBLADDER AND BILE DUCTS Waltman Walters B S, M D, M S in surgery, Sc D, F A C S Head of Section in Division of Surgery, Mayo Clinic, Professor of Surgery Mayo Foundation (University of Minnesota), and Albert M Snell, B S, M D, M S in Medicine, F A C P, Head of Section in Division of Medicine, Mayo Clinic, Professor of Medicine, Mayo Foundation (University of Minnesota) Cloth Pp 645, with 342 illustrations Price \$10 Philadelphia W B Saunders Company

## CHANGES IN THE ELASTICITY OF THE AORTA WITH AGE

JOSEPH KRAFKA JR., M.D.

AUGUSTA, GA

A series of investigations on the elasticity of the aorta has definitely established the curve of extensibility to be an exponential curve with the hollow at the 50-100 Gm tension level (Krafka<sup>1b</sup>). This is in keeping with the observations of Yater and Birkeland,<sup>2</sup> who noted that the greatest proportional stretch for strips was at the 50-100 Gm level. The same general phenomenon may also be seen in the curve showing the relation of volume to pressure as given by Wiggers.<sup>3</sup>

That there is a shift in the elasticity of the aorta with age is a generally accepted fact. Some investigators have claimed that this is not a progressive phenomenon, but that there is an increase in distensibility from birth to the twentieth year with a gradual decline from that time to old age. It is of interest to note that of 100 specimens studied by Yater and Birkeland, only 2 were from the 0-20 year age group, hence the conclusion is hardly justified. In any event, the increase in extensibility remains unexplained.

Loss in elasticity from maturity to senescence is considered to have an etiologic factor in (1) fibrosis, (2) loss in muscle tonus and (3) degeneration of elastic fibers. Since three separate histologic elements are involved, both quantitatively and qualitatively, the problem becomes complex in its variety of possible combinations leading to the same end result. An estimation of the relative values of each factor is possible only after a careful analysis of the elongation curve into the separate roles of muscle, elastic tissue and collagenous fibers.

Analysis of the elongation curve by the method of comparative elastic moduli indicates that the hollow portion of the curve is the region

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From the University of Georgia School of Medicine

This series of investigations was made possible by grant 469 of the Committee on Scientific Research of the American Medical Association

1 Krafka, J., Jr. (a) *Arch Path* **23** 110, 1937, (b) *Am J Physiol* **125** 1, 1939

2 Yater, W. M., and Birkeland, J. W. *Am Heart J* **5** 781, 1930

3 Wiggers, C. J. *Am J Physiol* **123** 644, 1938

of action of muscle and elastic fibers, while the straight line portion is due to the higher moduli of the collagenous fibers (Krafka<sup>11</sup>) Furthermore, normal diastolic and systolic pressure equivalents fall on the hollow of the curve (Krafka<sup>1</sup>) With these points in mind, it is

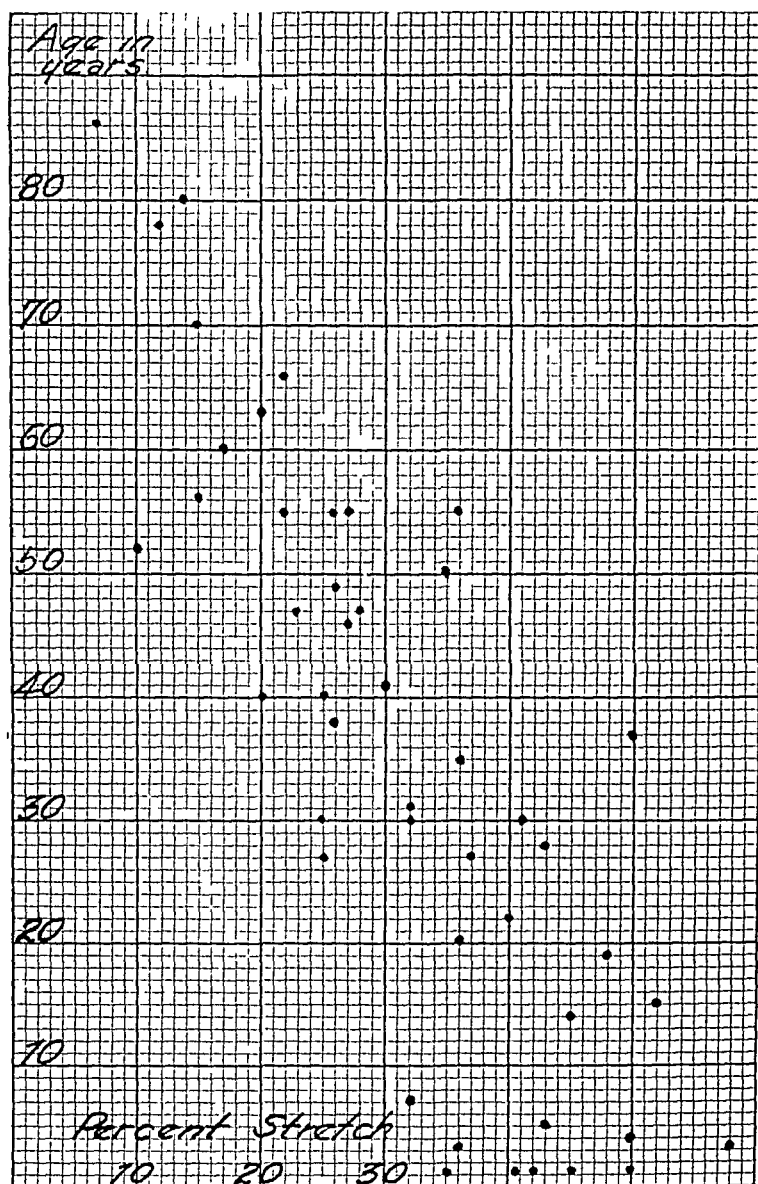


Chart 1—Scatter diagram showing percent stretch of aortic strips for the 100 Gm tension level. Note the marked decrease with age.

possible to evaluate the relative roles of the three histologic elements in the loss of elasticity of the aorta with age.

## METHODS

Elongation curves for longitudinal strips of aortas secured at autopsies were made on the recording seismograph as described in a

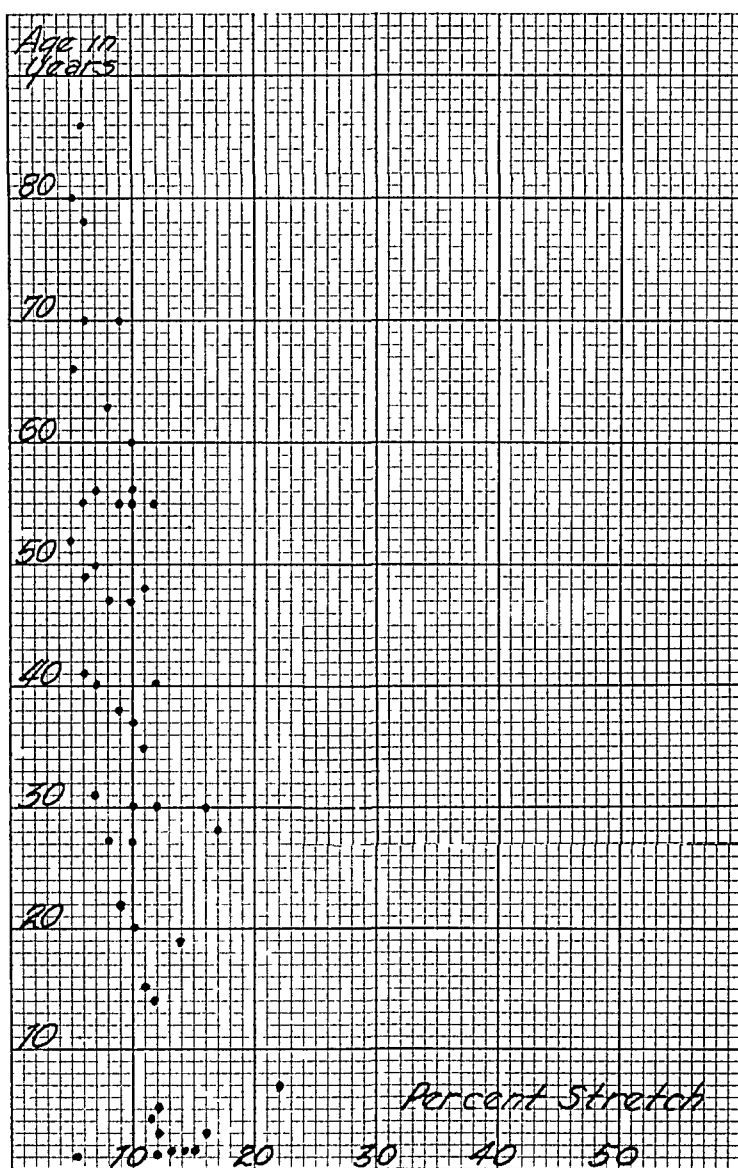


Chart 2—Scatter diagram showing percent stretch of aortic strips for the 200-100 Gm tension level. Note the gradual decrease with age.

previous paper (Krafka<sup>1b</sup>). From these curves, percent extensibility for 100 Gm tension was measured, and the data recorded as a scatter-gram (chart 1). Measurements were next made for the additional extension at from 100 to 200 Gm tension, i.e., for the straight line

portion of the curve These values are presented in a second scattergram (chart 2)

From the scattergrams several facts are at once apparent 1 There is no increase in distensibility from birth up to the twentieth year

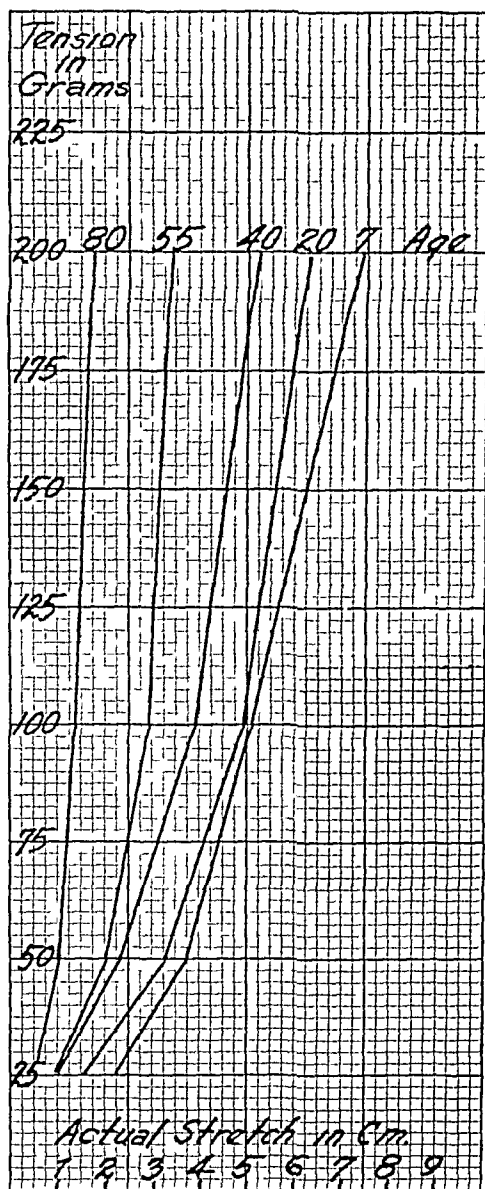


Chart 3—Serigrams of standard strips of human aortas 10 cm long and 5 mm wide Note the loss of elasticity with age

2 There is a gradual loss in elasticity with age throughout life in (a) the hollow portion of the curve and (b) the straight line portion of the curve 3 The difference, however, is not as marked for the straight line portion of the curve as it is for the hollow portion of the curve

The same phenomena are apparent from a comparison of the elongation curves for the various ages as given in chart 3, and from the data in the table

*Relationship of Percental Stretch of 10 Cm Strips of Human Aorta at Various Ages in Man (New Series)*

Age, Yr	Thickness, Mm	Percental Stretch at Given Number of Grams				Prevailing Blood Pressure
		50	100	200	200 100	
7	1.8	39	51	75	24	
20	2.6	35	51	64	13	
31	2.6	20	32	42	10	275/140
37	2.9	23	36	47	11	140/ 90
37	1.76	35	50	60	10	290/145
40	2.88*	20	40	53	13	195/130
41	3.18	22	30	35	5	105/ 75
47	2.34	18	27	35	8	230/160
48	2.46	20	28	30	11	145/ 83
55	2.90*	20	28	35	7	200/120
55	2.92*	24	36	48	12	175/ 95
80	2.36*	10	14	18	4	180/110

\* The aorta was markedly sclerotic

#### COMMENT

A general loss in the elasticity of the aorta has frequently been interpreted as due to an increase in the number of collagenous fibers. This point is significant in my theory of intimal herniation as the principal mechanical factor in the production of sclerosis (Krafka<sup>11</sup>). This concept, however, requires some alteration, since it is at once evident that a progressive loss of elasticity in the upper portion of the curve should not occur unless there is (1) a definite change in the elastic properties of the collagenous fibers or (2) a change in the architecture of the wall. A mere increase in the number of fibers in itself would fail to produce the aging effect.

A significant observation of Yater and Birkeland has a bearing on this point. Although they did not record their data, they concluded that there is no direct correlation between the thickness of the wall with age and loss in elasticity. The same fact was noted in the present series. For example, at the age of 80 years the thickness of the wall was 2.36 mm, at 55 years, 2.92 mm, at 41 years, 3.18 mm. Comparison of the elastic moduli shows that loss in elasticity is not primarily due to an increase in white fibers.

Another consideration enters the problem. Hallock and Benson<sup>5</sup> reported that the volume of segments of the aorta under pressure of

5 Hallock, P., and Benson, I. C. J. Clin. Investigation **16** 595, 1937

100 mm of mercury increases with increasing age. Thus the volume per centimeter of length at 100 mm pressure at the age of 20 years is 2.40, at 50 years, 2.60, at 78 years 3.20. This relationship may be accounted for on the simple assumption of a relaxation of the tube due to the failure of the musculoelastic tissue to maintain its tonus, which throws the entire recoil resistance on the white fibers. This in itself may produce an actual thinning and at the same time increase the modulus of elasticity. Hallock and Benson<sup>1</sup> considered the increase in volume to be due to an actual breakdown and disappearance of the elastic fibers, as established by Zon, but sections of aorta at old age still show an appreciable amount of elastic fibers.

Thinning of the aortic wall has been previously postulated on theoretic grounds (Kiafka<sup>1a</sup>) to be an adaptive mechanism which, by changing the relationship of the thickness of the wall to the diameter of the vessel, attempts to reestablish the original elasticity.

The key to the analysis of the problem was incidentally secured from tests of aortic strips before and after putrefaction (Kiafka<sup>1b</sup>). The immediate effect of putrefaction is to decrease the elastic modulus. But when calculations are made by the incremental method that bears directly on the collagenous fibers, the distended strips show the modulus of white fibers. In these tests an intact strip of aorta, 10 cm long, was tested and after putrefaction for twenty-four days, the same strip was fixed in the clamps of the seriograph at the points of the initial test. When the strips were fastened in the clamps with an initial state of no tension, instead of relaxed tension, a typical straight line curve was obtained.

Anomalous as it seems, loss of tone by relaxation of muscle elastic tissue thus increases the elastic modulus and at the same time increases the volume.

As evidence that the seriograph method is adaptable to this problem, calculations of the percental stretch of the wall based on the pressure-volume relationships of Hallock and Benson<sup>1</sup> give values which are closely comparable to those for the stretch observed with equivalent tensions on the seriogram. Thus for the age of 20 years the calculated stretch for 200 mm is 32.6 per cent, while the measured stretch on the seriogram is 39 per cent. For 78 years the total stretch calculated is 11.2 per cent, for 80 years on the seriogram it is 5 per cent.

These relationships warrant the conclusion of Hallock and Benson<sup>4</sup> that in old age the aorta assumes the role of a capacity chamber for the reception of cardiac output without due strain.

If a comparison is now made between the respective stretches for the ages 20 and 80 years, it will be seen that practically the entire loss of elasticity of the aorta may be accounted for on the basis of a relaxation

of the musculoelastic elements effective in the hollow portion of the curve. Thus the total stretch at 20 years is 64 per cent and that at 80 years is 18 per cent, with a difference of 46 per cent. This is a close approximation of the stretch at 20 years at 100 Gm., namely, 51 per cent (table).

A comparison of the 200-100 Gm. ranges for the ages 20 years and 80 years gives the values 13 per cent and 4 per cent, respectively. Hence the difference, 9 per cent, may be taken as the loss in elasticity due to a fibrosis factor. This compared with the 46 per cent would mean an approximate ratio of 1 to 5. Or stated as a generality, 80 per cent of the loss in elasticity of the aorta with age is due to failure of the musculoelastic system, 20 per cent, to fibrosis.

In conclusion, the statement may again be emphasized that sclerosis and loss in elasticity are not entirely correlated. The aorta may lose its characteristic as a rebound tissue without evident sclerosis, and localized sclerosis may exist without evident loss in elasticity.

A question is frequently asked as to the therapeutic application of the study of elasticity. The answer is that the present practice of rest in aortic dysfunction is entirely rational. As to medication designed to reestablish elasticity of the aorta, a search should be made for a smooth muscle tonic which at the same time would not affect the tonus of the heart.

#### CONCLUSIONS

1. Loss in elasticity of the aorta with age results from two factors (a) distensibility, 80 per cent, and (b) fibrosis, 20 per cent.
2. Increase in distensibility from birth to maturity is not established.
3. No direct correlation exists between elasticity, blood pressure, sclerosis and thickness of the aortic wall.



# SIMMONDS' DISEASE (PITUITARY CACHEXIA) IN AN AGED MAN WITH DEMENTIA PRAECOX

MYRIELLE M CANAVAN, M D  
BOSTON

In an autopsy on a man 72 years of age, who had died about three months after fracturing a femur, it was noted that the subject was greatly emaciated. The skin literally just covered his bones, and while his weight had not been recorded since the injury, he had weighed just previously to the injury 138 pounds (62.5 Kg). The dead body would not have registered more than 90 pounds (41 Kg) and his death had been expected every day for six weeks from sheer weakness, though he had taken food fairly regularly.

The patient suffered from dementia praecox and had been shifted from one mental disease hospital to a second and then to a third, where he had spent the last twenty-eight years being occupied when able, in light ward work. In July 1932 he was put to bed because of edema of his legs, and this subsided. On August 1 he had in some way fractured his left femur and remained in bed, his color, strength and weight decreasing until October 20, when he died.

The body measured 178 cm in length, there was little hair on the head, in the axillae or over the pubis. This would agree with Berman's<sup>1</sup> idea of pituitary inactivity, since he stated that the removal of the anterior lobe of the pituitary results in loss of hair.<sup>2</sup>

When the pituitary was removed and held by a dual tag in forceps, it resembled an edematous string.

The chronic lesions found were hypertrophy of toe nails, enlarged mesenteric lymph nodes, diverticula of the jejunum, a few plaques in the coronary arteries, aorta and basal vessels, a small, flabby heart, a large gallbladder, a *very small stomach* (not much larger than the gallbladder), hypertrophy of the prostate, atrophy of the testicles and thickening of the pia-arachnoid. The acute changes were pressure sores on one ankle, fluid in the pleural cavities and bronchopneumonia.

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From the Pathological Laboratory of the Massachusetts Department of Mental Health

1 Berman, L. The Glands Regulating Personality, ed 2, New York, The Macmillan Company, 1928, chap 8

2 I have observed that pubic hair shows marked differences in age groups between puberty and 25 years this hair is often glistening, curly and abundant. After 40 the hair is dryer and more nearly straight, streaks of gray increasing with age, until at 90 it is represented by a few thin spears of white hair. This loss is more marked in men than in women.

That he had only mild thickening of the blood vessels and no tuberculosis or malignant growth to account for the emaciation was of interest, therefore the association of this loss of weight with the edematous small pituitary was unavoidable. Correspondence with the superintendents of Massachusetts state hospitals brought no reports of any clinical manifestations of pituitary lack in their patients.

In an active pathologic service with the Massachusetts Department of Mental Health for over twenty years I have never seen a pituitary

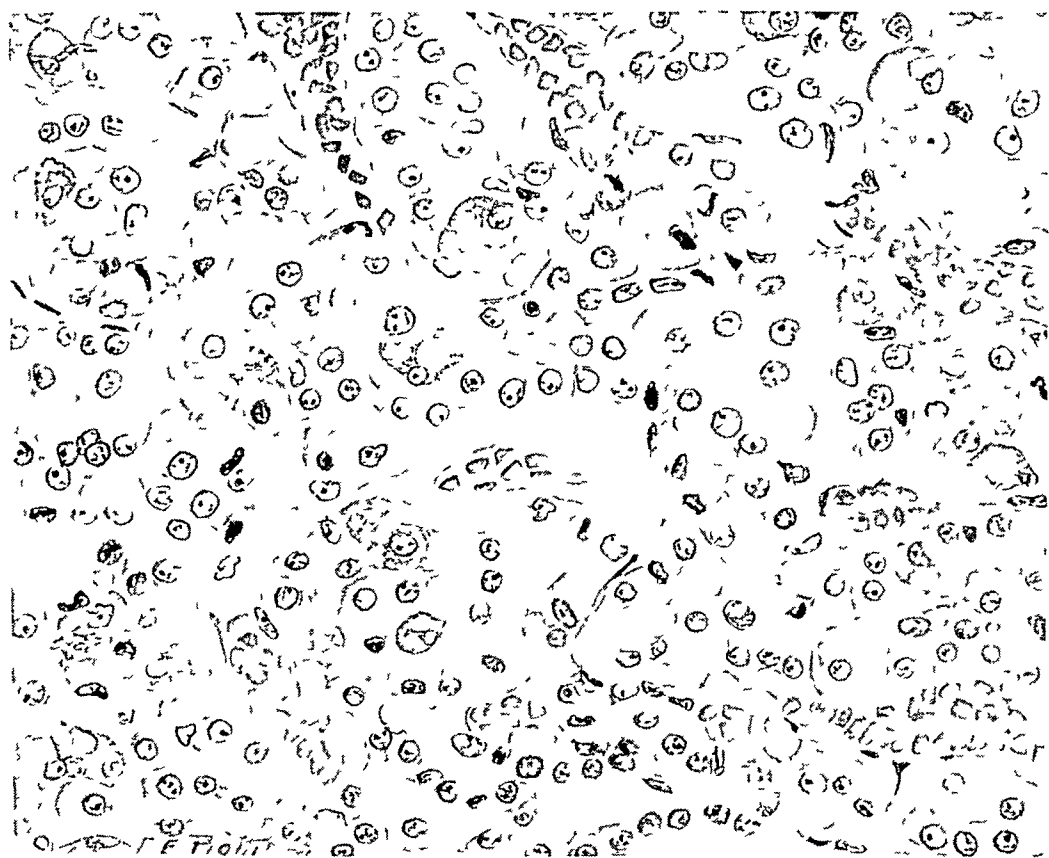


Fig 1—Normal pituitary. Note numerous sinusoids filled with red blood cells. There is a suggestion of acinar arrangement. The abundant acidophils vary in size and in depth of protoplasmic coloring. The chromophobes are with or without protoplasm. The basophilic cells are large and finely granular, with nuclei often eccentric, and show the characteristic vacuole near the nucleus. Hematoxylin and eosin,  $\times 540$ .

of similar gross appearance. Microscopically, the anterior lobe consisted chiefly of collapsed and dull polychromatic edematous cells and showed many free nuclei and shadows of cell outlines. The acidophils showed marked vacuolation of the cytoplasm, in 50 fields an average of 2 solidly granular acidophils was found. There was definite paucity

of basophils. The chromophobes appeared normal. There was no increase of interstitial tissue, and the walls of the sinusoids were nearly in apposition.

Silver,<sup>3</sup> in a review of the literature to 1933, stated that persons suffering from Simmonds' disease show certain characteristic clinical and pathologic features. Their ages vary from 9 to 69. Their disease occurs after pregnancy, infection, accidental injury, alcoholism or an unknown cause, and the duration varies from seven months to

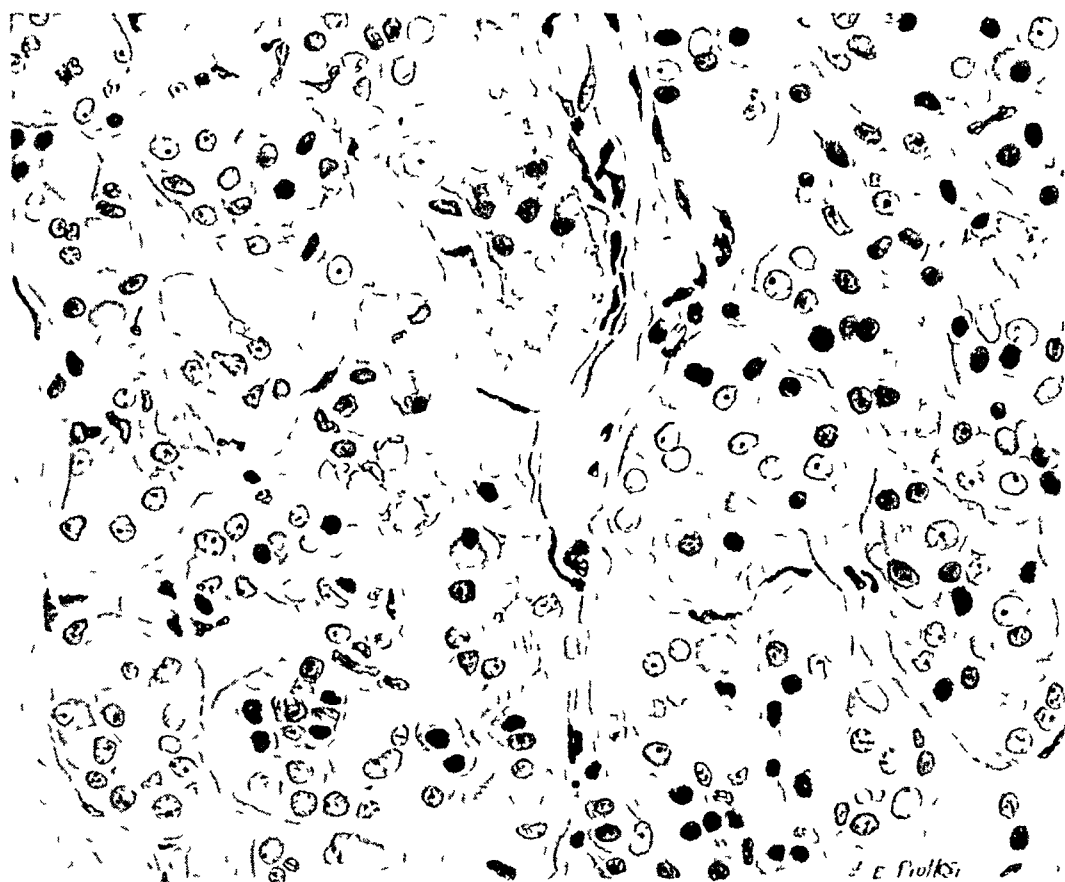


Fig 2—Pituitary of a patient with Simmonds' disease. The collapsed sinusoids contain no red blood cells, and there is proliferation of their endothelial lining. There are no basophils. The majority of the cells are vacuolated acidophils. Hematoxylin and eosin,  $\times 540$ .

forty-four years. They show premature aging, loss of weight, strength, libido, teeth, pubic and axillary hair, and atrophy of the genitalia and of the jaw. Besides these signs, hypotension, hypothermia, eosinophilia and anemia are found.

The patient under consideration was 72, his signs came on after fracture of a femur, and their duration was eleven weeks. He pre-

sented some of those changes given in Silver's review, namely, cachexia, marked asthenia, loss of pubic and axillary hair and atrophy of the testicles, and he was very pale. His advanced age, in contrast to those mentioned by Silver, makes the clinical signs, with the exception of the marked loss of weight, less significant.

The pathologic changes (condensed from Silver's review) are smallness of viscera and atrophy of the endocrine glands in some cases. Changes in the pituitary are prominent. These include loss of weight of the gland and smallness of the gland, with fibrous, cystic, tuberculous or hemorrhagic lesions in the anterior lobe. The patient concerned in this report showed the small stomach, the atrophy of the testicles and the marked degeneration in the anterior lobe of the unweighed gland.

#### SUMMARY

The patient was a 72 year old man with dementia praecox. Marked emaciation occurred after a fracture of the femur. The duration of Simmonds' disease was eleven weeks. Grossly, the pituitary was small and edematous. Microscopically, collapse of the sinusoids was seen as well as varying degrees of degeneration of the cells in the anterior lobe, particularly of the acidophils.

# PRIMARY AMYLOID DISEASE OF THE MYOCARDIUM AND BLOOD VESSELS

REPORT OF A CASE WITH DEATH FROM MYOCARDIAL FAILURE

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Passed Assistant Surgeon United States Public Health Service

DETROIT

The literature records few cases in which death was due to cardiac failure brought about by deposition of amyloid in the myocardium. Kerwin<sup>1</sup> collected 5 cases and reported 2 additional cases of his own. He stated that Wilks,<sup>2</sup> in 1856, recorded the first case. Budd<sup>3</sup> reported a case, that of a 75 year old man who died of carcinoma of the prostate, the heart showed advanced myocardial amyloidosis. Larsen,<sup>4</sup> in addition to a case observed by himself, in which there was definite myocardial failure (included in Kerwin's review), mentioned a case observed by Beneke and Bonning<sup>5</sup> that of a man aged 70 years who entered the hospital in a moribund condition with a diagnosis of bronchitis, extensive amyloid deposition was found in the heart, venae cavae and lungs.

Bannick and his co-workers<sup>6</sup> reported diffuse amyloidosis occurring in a 44 year old man who had died of a combination of profound renal, adrenal and hepatic insufficiency. In addition to large amounts of atypically staining amyloid deposited in these organs and in the spleen, the walls of many blood vessels showed acellular hyaline material<sup>7</sup> which was stained by congo red. The myocardial muscle cells revealed marked atrophy and the stroma was increased because of narrow bands of hyaline substance, which also had an affinity for congo red. In some areas muscle fibers had disappeared entirely.

Primary systemic amyloidosis is a rare entity. In their recent review Koletsky and Stecher<sup>7</sup> found 22 recorded cases and added a case.

In the case to be reported, cardiac amyloidosis appeared to have been responsible for myocardial failure and death. As there was no other associated chronic disease, the condition must be classed as primary.

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From the Laboratory of the United States Marine Hospital

1 Kerwin, A. J. *J. Lab. & Clin. Med.* **22**: 255, 1936.

2 Wilks, S. *Guy's Hosp. Rep.* **2**: 105, 1856.

3 Budd, J. W. *Am. J. Path.* **10**: 299, 1934.

4 Larsen, R. M. *Am. J. Path.* **6**: 147, 1930.

5 Beneke, R., and Bonning, F. *Beitr. z. path. Anat. u. z. allg. Path.* **44**: 362, 1908.

6 Bannick, E. G., Berkman, J. M., and Beaver, D. C. *Arch. Int. Med.* **51**: 978, 1933.

7 Koletsky, S., and Stecher, R. M. *Arch. Path.* **27**: 267, 1939.

## REPORT OF CASE

M T C, a white man aged 56, married, a veteran of the Spanish-American War, was admitted to the hospital Aug 2, 1938. He had not worked for several years but formerly was employed as a meat cutter in a grocery store. With the exception that his mother died of tuberculosis, his family history was not significant. His personal history revealed that he had had an attack of acute bronchitis twenty years ago. He used alcohol in moderation and until five weeks before admission had used tobacco. He stated that his health had been fairly good until about eight months before admission, when he became dyspneic on exertion. Symptoms had become progressively worse. About one month before admission he had spent ten days in another hospital, where a thorough study was made, and the attacks of dyspnea were thought to be due to bronchial asthma. However, based on electrocardiographic studies, an additional diagnosis of anterior myocardial infarction (old) was made. At the same time he was treated for mycosis of the finger nails and toe nails. During the month prior to admission here there had been a productive cough, orthopnea and swelling of the ankles.

*Physical Examination*—The patient was a fairly well developed middle-aged man, weak, somewhat cyanotic and apparently acutely ill. There were crepitant and coarse rales throughout the chest and suppression of breath sounds over the base of the right lung. The heart was not enlarged to percussion, the rhythm appeared to be tic-tac and no murmurs were heard. The blood pressure was 92 systolic and 78 diastolic. The peripheral vessels were thickened and tortuous. No peripheral edema was present. The temperature was 37 C and the pulse rate 100. The urine showed a faint trace of albumin, and the specific gravity was 1.026. The Kahn test of the blood was negative. The erythrocytes numbered 4,550,000, and the leukocytes 9,000 per cubic millimeter. The hemoglobin content was 75 per cent. A differential count revealed 85 per cent neutrophils, 11 per cent lymphocytes and 4 per cent monocytes. Roentgen examination of the chest showed bilateral irregularity of the diaphragm due to pleural adhesions, thickening of the pleura on the right lower region of the chest, thought to be associated with some pleural effusion, and compression atelectasis of both lower pulmonary fields. The heart was not enlarged. An electrocardiogram exhibited extremely low voltage throughout.

*Course*—Under a regimen of rest in bed, digitalis and sedation the patient temporarily became more comfortable. It was soon the opinion of his physicians that the dyspnea was due to myocardial degeneration and not to bronchial asthma. About one month after admission he was allowed to be up for short periods. Later, however, his dyspnea and nocturnal wheezing returned, his ankles showed edema, and a roentgen picture of the chest revealed bilateral hydrothorax. Repeated thoracenteses were done on alternate sides, with recovery of approximately 1,000 to 2,000 cc of clear fluid of a specific gravity of about 1.012. The patient complained persistently of flatulence and vague precordial distress. Mercurial diuretics and ammonium chloride were also administered from time to time. The blood pressure taken three months after admission was 80 systolic and 60 diastolic. He became progressively weaker and died March 10, 1939, about seven months after his final hospitalization and fifteen months after the onset of severe symptoms.

*Autopsy*—The autopsy began two and one-half hours post mortem.

Externally the body presented no abnormalities with the exception of scaliness and partial loss of several finger nails. No lesions of the skin were seen.

There was a small amount of clear fluid in the abdominal fossae. The pleural cavities contained much fluid, that on the left was clear and yellowish, but that on the right was turbid and dark red. Both cavities showed many adhesions between the visceral and the parietal layer of pleura.

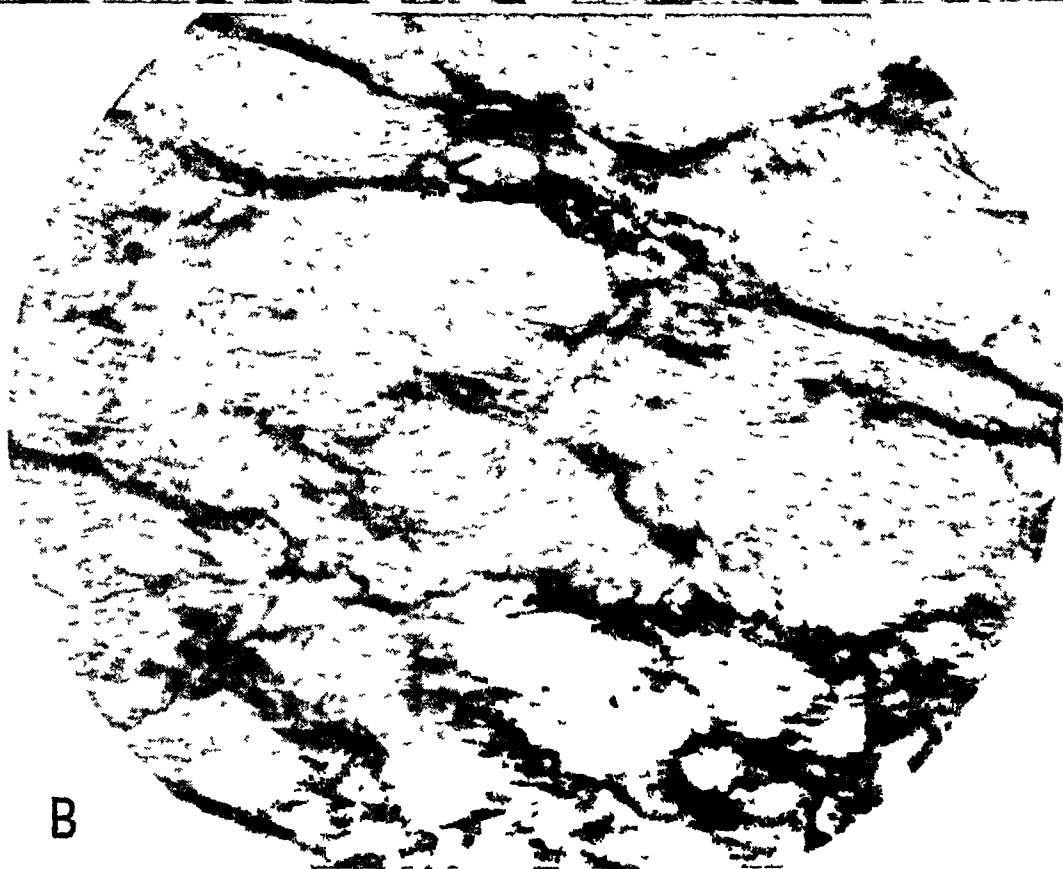
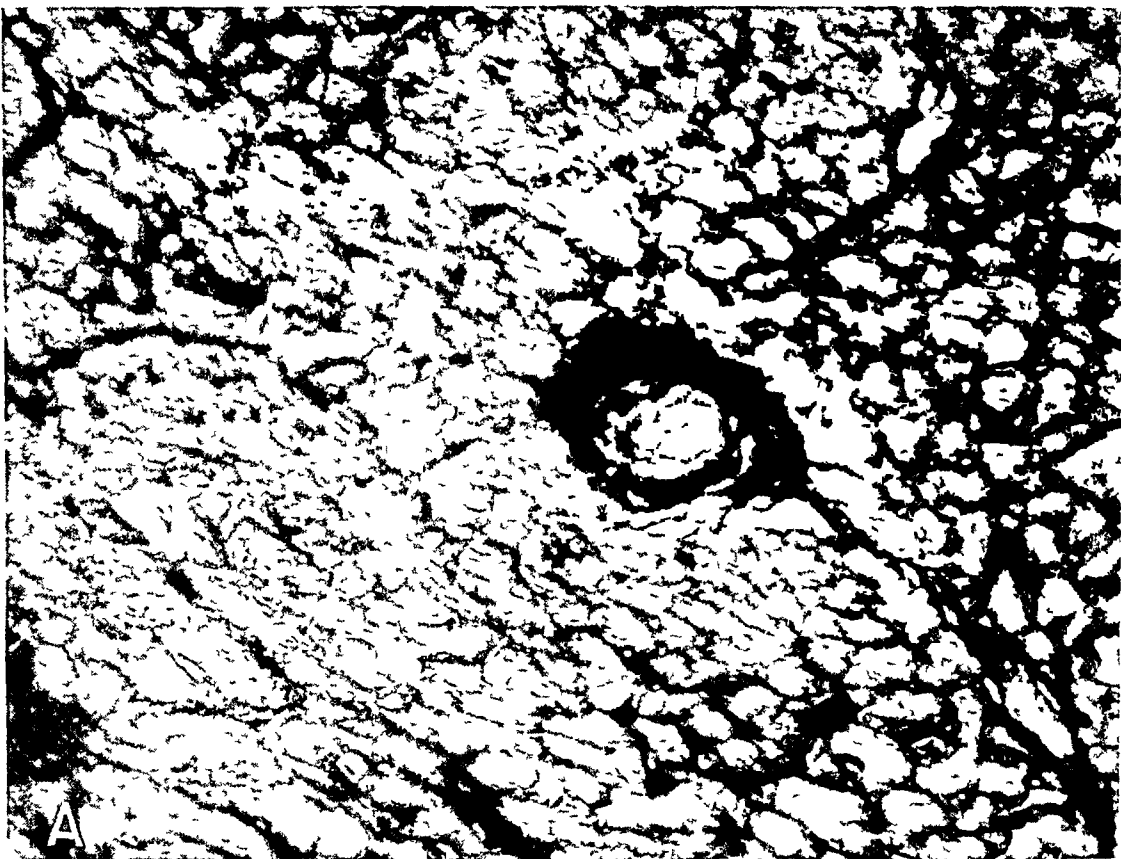


Fig 1—*A*, interstitial infiltration by hyaline material and deposition of amyloid in an arteriolar wall in the left ventricle of the heart, fast green (Masson's method),  $\times 150$  *B*, higher magnification of *A*,  $\times 600$

The heart, which weighed 400 Gm, was normally situated in the thorax. The greatest transverse diameter measured 14 cm. There were only a few cubic centimeters of pericardial fluid. The pericardium appeared normal. The right ventricle was slightly dilated and the left contracted. On section the myocardium of the right and left ventricles and interventricular septum was firm and its color pinkish red. The wall of the left ventricle measured 10 mm and that of

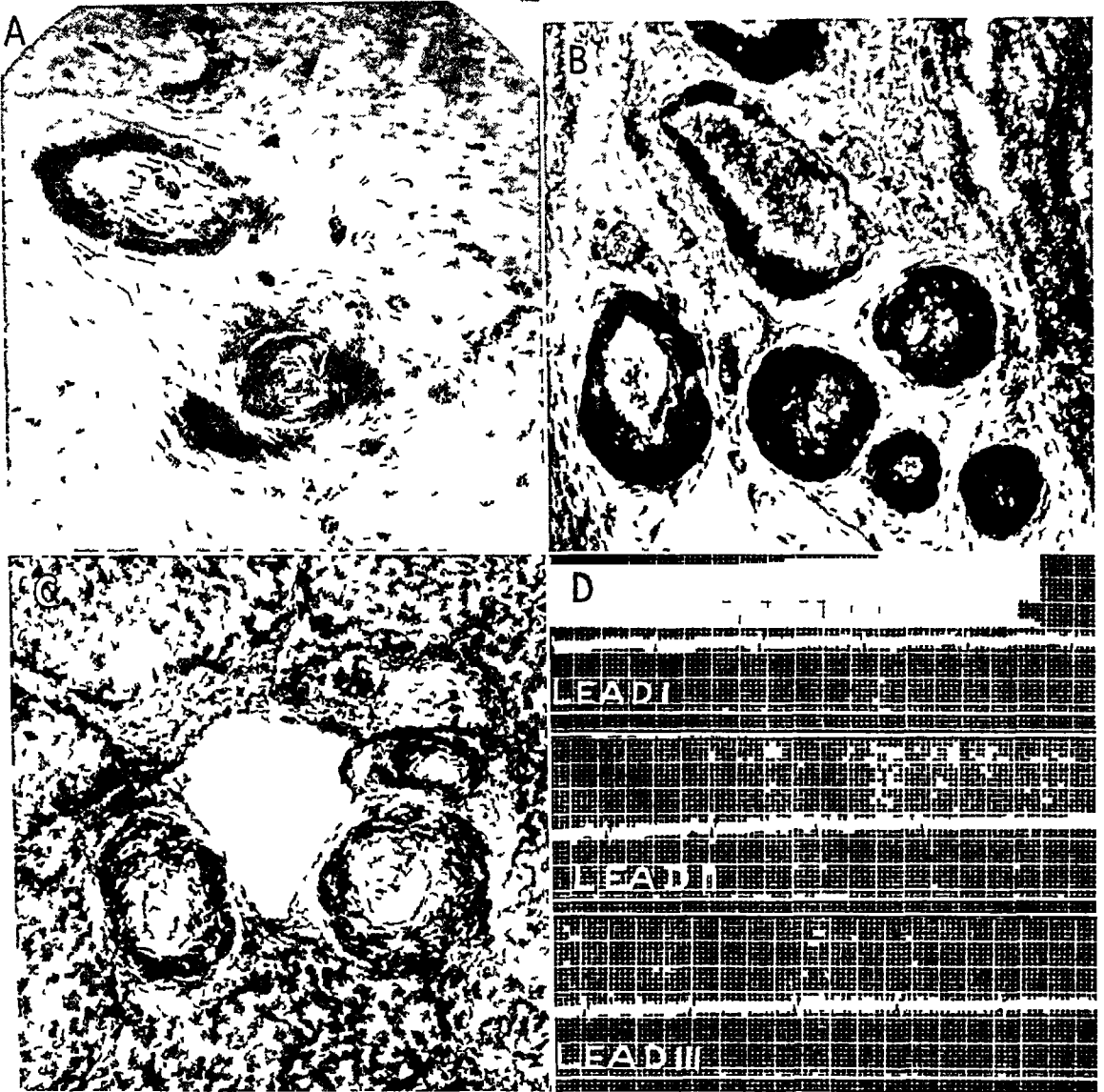


Fig 2—*A*, obliterative deposition of amyloid in the walls of small vessels in the left ventricle of the heart, gentian violet,  $\times 150$ . *B*, amyloid deposits in periadrenal arterioles and venules, methyl violet,  $\times 150$ . *C*, amyloid deposits in thick-walled interlobular vessels of the liver, methyl violet,  $\times 90$ . *D*, electrocardiogram made Sept 6, 1938

the right ventricle 2 to 4 mm in thickness. The leaflets and rings of the various valves appeared normal. The coronary arteries were patent and presented no significant sclerotic changes. In the right auricular appendage were numerous soft friable thrombi.



The abdominal aorta exhibited a few intimal atheromatous plaques. Changes in other levels were minimal. The left common iliac artery was plugged by a friable soft gray clot, but the extremity showed no evidence of infarction. There was occlusion of the branch of the pulmonary artery supplying the lower lobe of the right lung, with resulting massive infarction. A small infarct was also present in the inferior border of the upper lobe of the right lung. The liver, which weighed 1,000 Gm., showed centrilobular congestion. The right kidney presented several small infarcts. There was a single small infarct in the spleen.

The other organs presented no abnormalities. While the autopsy explained the death on the basis of massive pulmonary infarction, there was no anatomic observation to explain the cardiac failure.

*Microscopic Examination*—Sections of the left ventricle of the heart showed significant amounts of opaque amorphous substance deposited in the epicardium and myocardium. It was most conspicuous in the media and perivascular zones of the small blood vessels but also was seen as focal aggregates replacing muscle fibers or as pericapillary thickenings diffusely spread between individual fibers. The muscle fibers often appeared fragmented, as if being constricted or choked by the infiltrating substance. The media of the numerous arterioles and venules infiltrated by the substance showed much thickening and loss of recognizable muscle fibers. The lumens of the smaller vessels were markedly stenosed. The vascular endothelium was preserved.

The substance was stained bright yellow by Van Gieson's solution of trimrophenol and fuchsin, pale blue by Romanowsky's stain,<sup>8</sup> pink by hematoxylin and eosin (frozen section), pink by congo red and pale to dark green by fast green used instead of light green in a modification<sup>8a</sup> of Masson's trichrome method. With dilute iodine on frozen sections, the thicker deposits became pale brown, and after exposure to 5 per cent sulfuric acid the color changed to greenish black. All stains were made on material fixed in solution of formaldehyde, U. S. P., and with the exception of those on frozen sections were on paraffin-embedded material. With methyl violet and gentian violet the deposits in the walls of blood vessels stained the characteristic reddish violet of amyloid. The fine deposits about the individual fibers occasionally showed metachromatic staining. However, with the nonspecific stains the interstitial thickening was very conspicuous. This infiltrate was stained yellow by Van Gieson's solution of trimrophenol and fuchsin. The staining reactions indicated that at least the denser deposits of the substance belonged to the amyloid group, and that term will be used in the descriptions which follow.

Sections taken from the interventricular septum, the right ventricle and the auricles showed similar infiltration by amyloid. In the septum there was a little interstitial fibrosis.

The other organs studied, including the lungs, liver, kidneys, adrenals, urinary bladder, prostate, seminal vesicles, spleen, pancreas, tracheobronchial lymph nodes, thyroid, diaphragm and femoral nerve, showed marked infiltration and thickening of the media of arterioles and venules by amyloid. However, no significant amounts were deposited about the sinusoids of the liver, around the glomerular capillaries of the kidneys, between the cords of the adrenal cortex and in the corpuscles of the spleen. The diaphragm showed a moderate number of fibers which had an opaque appearance, had lost striations and were relatively acidophil. There was also a little poorly defined amorphous substance between the fibers.

8 Lillie, R. D., and Pasternack, J. G. *J. Tech. Methods* **15**: 65, 1936.

8a Goldner, J. *Am. J. Path.* **14**: 237, 1938.

Though the walls of some of the larger veins exhibited small quantities of amyloid, the larger arteries showed none except a little deposited in the vasa vasorum

The following diagnoses were made: generalized vascular amyloidosis, cardiac vascular and interstitial amyloidosis, with myocardial degeneration, chronic passive congestion of the lungs, liver and spleen, infarction of the lungs, kidneys and spleen, thrombosis (or embolism) of the left common iliac artery

#### COMMENT

The myocardial failure in this case appears to have been due to the obliterative stenosis of the smaller divisions of the vascular tree by the amyloid deposits in their walls. The circulation to the myocardial fibers was further impaired by the interstitial deposition of hyaline material. Though there were scattered small nodules of amyloid replacing muscle fibers, there was not shown the extensive nodular infiltration of the myocardium reported by several authors.

In commenting on his case, Laisen<sup>4</sup> stated that the amyloid was always deposited about capillaries and venules of the heart and that it was not found in the walls of the coronary arteries. He expressed the belief that the necrosis and loss of muscle fibers had been due to the obliteration of the capillaries and venules. In the present case the arterioles and venules were equally affected. Arteriole obliteration very probably caused the development of myocardial failure and death without gross nodular amyloidosis of the myocardium.

The widespread deposits in the walls of the smaller vessels in all organs studied is of interest. It is noted that the usual deposition in the liver, spleen, adrenals and kidneys did not take place. Koletsky and Stecher<sup>7</sup> emphasized the observation that in primary amyloidosis, instead of the deposits in the usual organs, the tongue, heart, stomach, intestine and skeletal muscle are most frequently affected.

The hyaline material found enveloping the myocardial capillaries and muscle fibers was easily demonstrated by nonspecific stains, but only rarely did it show any metachromatic staining characteristics when stained by methyl violet or gentian violet. Kerwin<sup>1</sup> mentioned the difficulty experienced in obtaining typical amyloid reactions in the heart in his case and quoted Lubarsch<sup>9</sup> as stating that there may be a failure in usual staining reactions in atypical amyloidosis. He also calls attention to a difficulty reported by Smetana<sup>10</sup> in obtaining stains of recently formed amyloid produced experimentally.

#### SUMMARY

The case of a 56 year old white man who died of myocardial failure and cardiac asthma is reported. Autopsy showed stenosing amyloidosis

<sup>9</sup> Lubarsch, O. *Virchows Arch f path Anat* **271** 867, 1929

<sup>10</sup> Smetana, H. *Bull Johns Hopkins Hosp* **37** 383, 1925

of the small coronary vessels and interstitial deposition of a hyaline substance in the myocardium as well as generalized amyloidosis of the small arteries and veins. There was no associated chronic disease.

Dr. W. W. Nesbit and Dr. H. R. Ostrander, of the United States Marine Hospital, Detroit, supplied the clinical data on the case reported, and Dr. Edgar H. Norris, professor of pathology, Wayne University Medical School, Detroit, gave aid in obtaining the photomicrographs.

# WAVE MECHANICS OF SMOOTH MUSCLE ACTION

## XV EXPERIMENTAL MULTIPLE REFLECTIONS BETWEEN INTESTINAL LIGATURES TRANSFORM TRAVELING INTO STATIONARY MICROPRESSURE WAVES IN SMOOTH MUSCLE

EBEN J CAREY, M D

MILWAUKEE

The physical nature of the microscopic structural changes associated with the physiologic and pathologic contractions of smooth muscle is unknown. Excellent observations on fixed material have been recorded by McGill<sup>1</sup> and like observations on living isolated smooth muscle cells by Margaret and Warren Lewis.<sup>2</sup> McGill's description of swollen contraction nodes was confirmed by Lewis and Lewis. They stated, furthermore, that the narrow internodal region in the living cell is under longitudinal tension or stretch. The Lewises were unable to observe the nuclear changes within the active contraction nodes. This was probably due to the low refractive index of the nucleus as compared with that of the active living cytoplasm in the contraction node. The physical significance, however, of the alternate nodes and internodes in contracting smooth muscle is unknown.

The object of this paper, therefore, is to present experimental evidence that points to an *associated micropressure wave mechanics of protoplasmic activity inseparable from the colloidal physical and chemical reactions of smooth muscle contraction*.

These explosive micropressure waves of confined colloidal chemical changes underlie both the gross and the microscopic structural displacement of living muscle protoplasm. The absence and the presence of pressure waves, therefore, determine the variable and reversible or irreversible differential spatial distribution and physical attributes of both the cytoplasm and the nuclei of smooth muscle during rest and motion. In resting smooth muscle these waves are relatively absent.

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From the Department of Anatomy, Marquette University School of Medicine.

These investigations were carried out with the aid of a grant for research to the Department of Anatomy of the Marquette University School of Medicine by the Committee on Scientific Research of the American Medical Association. A part of the histologic evidence presented in this paper was demonstrated at the April 1939 meeting of the American Association of Anatomists at Harvard University (Anat Rec **73** 11 and 62 1939).

1 McGill, C. Am J Anat **9** 493, 1909.

2 Lewis, M. R., and Lewis, W. H. Am J Physiol **44** 67, 1917.

During contraction the colloidal particles are aggregated in the regions of greatest intensity of the longitudinal waves, namely, the zones of condensation. The absence and the presence of the two regions of (1) nodal compressional condensation and (2) internodal tensional rarefaction of the longitudinal pressure waves determine the reversible alterations in the internal structure from the state of rest to that of motion respectively, of smooth muscle.

It is the gradient pressure and power of superposed multiple microscopic traveling and overlapping waves of internal compression associated with chemical reactions that push the bolus and partly determine the directional stabilization in the action of smooth muscle. This is in harmony with the metabolic gradient theory of Alvarez<sup>3</sup> regarding the directional stabilization of motion of the peristaltic waves of the intestines. The grossly visible propulsive peristaltic waves of the intestine are resultants of multiple overlapping and microcompressional waves by algebraic addition. By experimentally changing the resultant traveling into a spastic stationary system of intestinal peristaltic waves, by multiple reflections and constructive interference, between ligatures, the components of the micropressure waves become separated, fixed and oriented in space. The structural identification of the parts of the micropressure waves in smooth muscle under the described experimental conditions is therefore clearly evident in histologic preparations. An irreversible, fixed dynamic ileus of the intestinal segments is produced in these experiments.

#### MATERIALS AND METHODS

Sixty-five etherized guinea pigs, weighing about 250 Gm each, are used in these experiments. A midline ventral abdominal incision is made. The large and small intestines are exposed, with blood supply intact, and placed between gauze sponges saturated with Ringer's solution warmed to 37 C. Fecal pellets are gently pushed above and below the sites selected for encirclement of the large intestine with tightly tied catgut ligatures placed 1 inch (2.5 cm) apart. At variable sites between the ligatures 0.5 cc of Ringer's solution at 37 C is injected into the intestinal lumen in each of fifty segments, in 10 animals, 0.5 per cent acetylcholine in 0.5 cc of Ringer's solution is injected into the lumens of fifty segments, in 10 animals, 0.5 per cent pilocarpine in 0.5 cc of Ringer's solution is injected into the lumens of fifty segments, in 10 animals. A small crystal of barium chloride is placed on the external surface of each of twenty intestinal segments, in 5 animals.

As soon as the opacity indicating intestinal spastic contraction occurs between the ligatures, the segment is excised with the terminal ligatures and placed in Ringer's solution at 5 C for ten minutes to stop the active state of the muscle, it is oriented and tied on 1 by 3 inch (2.5 by 7.5 cm) microscopic glass slides, then fixed in a 10 per cent solution of neutral formaldehyde U.S.P. in 20 per cent dilution, for twenty-four hours. Pieces one-half (1.2 cm) inch long are

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<sup>3</sup> Alvarez, W. C. *The Mechanics of the Digestive Tract*, New York, Paul B. Hoeber, Inc., 1929, p. 55.

dehydrated in alcohol, cleared in xylene, embedded in paraffin and cut serially in both cross and longitudinal sections at 4 to 6 microns. The sections are variously stained, but hematoxylin and erythrosin give good uniform results.

For inactive controls 0.5 per cent atropine in 0.1 cc of Ringer's solution is injected into the lumens of fifty segments, in 5 animals. In addition controls are obtained by killing guinea pigs with ether. Then the animals are placed in a refrigerator at 5 C for twenty-four hours before the abdomen is opened and the intestinal segments excised for microscopic preparation. Irritability is therefore destroyed before the intestines are handled and placed in a fixative. Control traveling waves are obtained and fixed by injecting subcutaneously 5 cc of 1 per cent pilocarpine in Ringer's solution at 37 C in 5 etherized animals. The abdomen is then opened. The entire animal with stimulated intact intestines is submerged and fixed in solution of formaldehyde U. S. P. diluted 1:10, for twenty-four hours.

The external surface of the exposed intestinal segments between ligatures, with blood supply intact, is observed under Ringer's solution at 37 C in 20 animals, with the Ultrapac microscope and water immersion lenses. The various experimental procedures enumerated are repeated. Direct observations of the peristaltic waves through the serosa are made. The direct conversion of traveling peristaltic waves into a stationary system is observed between the intestinal ligatures in both the large and the small intestine.

The gizzard of the pigeon is studied in microscopic section in order to determine the histologic changes following inactivity and activity. The inactive gizzard muscle is obtained by killing the bird with magnesium sulfate, 2 Gm per kilogram of body weight. The birds die in one to three hours after receiving the injection. They are then placed in the refrigerator at 5 C for twenty-four hours prior to fixation. The active gizzard muscle is obtained by immersing the gizzard in Ringer's solution at 39 C for ten minutes prior to fixation.

#### EXPERIMENTAL RESULTS

The direct conversion of traveling propulsive peristaltic waves into short nonproductive stationary waves of spastic contraction is observed with the aid of the Ultrapac microscope on the external surface of the intestinal segments between the ligatures. Shortly after the injection of 0.5 cc of 0.5 per cent acetylcholine in Ringer's solution into the lumen of the segment short traveling incident waves radiate both ways from the site of the injection. These fine waves produce localized rhythmic contractions. Multiple reflections of the fine ripples occur back and forth between the ligatures.

This is comparable to observations made with the unaided eye on the external surface of the intestine by Engelmann,<sup>4</sup> Cannon,<sup>5</sup> Meek,<sup>6</sup> Hyman,<sup>7</sup> Child,<sup>8</sup> Alvarez,<sup>9</sup> and others. Alvarez used the word "metabolism" and the term "metabolic or physiologic gradient" in reference to

4 Engelmann, T. W. *Arch f d ges Physiol* **2** 259, 1869

5 Cannon, W. B. *Arch Int Med* **8** 419, 1911

6 Meek, W. J. *Am J Physiol* **24** 232, 1911

7 Hyman, L. H. *Biol Bull* **37** 388, 1919

8 Child, C. M. *Biol Bull* **39** 147, 1920

the directional stabilization of the peristaltic waves in the same sense as Hyman<sup>7</sup> and Child,<sup>8</sup> respectively, used them. They are indicators, respectively, of the sum of all the energy-producing and substance-producing processes and the differential rates of chemical change between two localities in space. Oxidative processes are eventually and ultimately associated with all of the other physical and chemical factors in the living protoplasmic system even though for short periods certain chemical reactions are anaerobic in muscle.

There is sudden cessation of intestinal movement when 0.5 cc of 0.5 per cent acetylcholine in Ringer's solution is injected into the lumen of a segment of the large intestine 1 inch long. Superpositional constructive interferences of incident and reflected waves produce a strong tonic contraction. When spasticity occurs, the intestinal segment immediately becomes opaque and relatively bloodless. The two intestinal ligatures act like minor obstacles and cause multiple reflections in a confined localized space of the intestinal segment. The shape of the intestinal segment after fluid has been injected into the lumen may be uniformly cylindrical, dumbbell, fusiform or Indian club shaped. This shape is dependent on the site and on the rapidity of the injection of fluid into the lumen between the two ligatures. Long overlapping constrictions of the traveling wave system are 1 to 8 mm in length. They have a relatively smooth external surface. When they are converted into the stationary system of waves, there is a subdivision into microscopic waves, which appear as convexities on the external surface of the intestine. These fine convexities in the spastic intestinal segments remain relatively fixed, and the distance from the crest of one convexity to the crest of the next is 10 to 120 microns.

#### HISTOLOGIC RESULTS

The inner, closely wound spiral muscle coat (Carey,<sup>9</sup> Reid, Ivy and Quigley<sup>10</sup>) is not as favorable a location in which to observe the histologic changes of smooth muscle contraction as the outer, open, elongated spiral layer. The reason for this is the difficulty of cutting the fibers of the inner layer parallel to their long axis. The muscle fibers of the outer layer are, therefore, relatively more longitudinal and parallel to the long axis of the intestine. The muscle fibers of the outer layer are, therefore, relatively more longitudinal and parallel to the long axis of the intestine. Cleancut patterns of alternate condensations and rarefactions of the cytoplasm and nuclei are best observed in longitudinal sections of the intestine (figs. 27, 28 and 29) as nearly parallel as possible to the longitudinal direction of the fibers in the outer muscle

9 Carey, E. J. *Anat. Rec.* **21**, 189, 1921.

10 Reid, P. E., Ivy, A. C., and Quigley, J. P. *Am. J. Physiol.* **109**, 483, 1934.

layer Since the cutting of the sections in a favorable plane is a trial and error proposition, many thousands of sections must be made and studied in order to obtain favorable patterns for record

Relatively resting smooth muscle is observed in cross (figs 1, 8 and 24) and longitudinal (fig 26) sections of the large intestine In the active muscle of the inner coat there are localized lateral expansions designated by arrows in figures 2, 3 and 4 Underlying these expanded areas are irregular alternate dark and light stripes of the cytoplasm The orientation of these stripes is better observed in the outer muscle layer (figs 27, 28 and 29) In the dark stripe many nuclei are round and uniformly deeply stained, whereas in the light one they are elongated and granular This becomes clear when the outer coat of muscle is observed in longitudinal sections of the intestine (figs 27, 28 and 29)

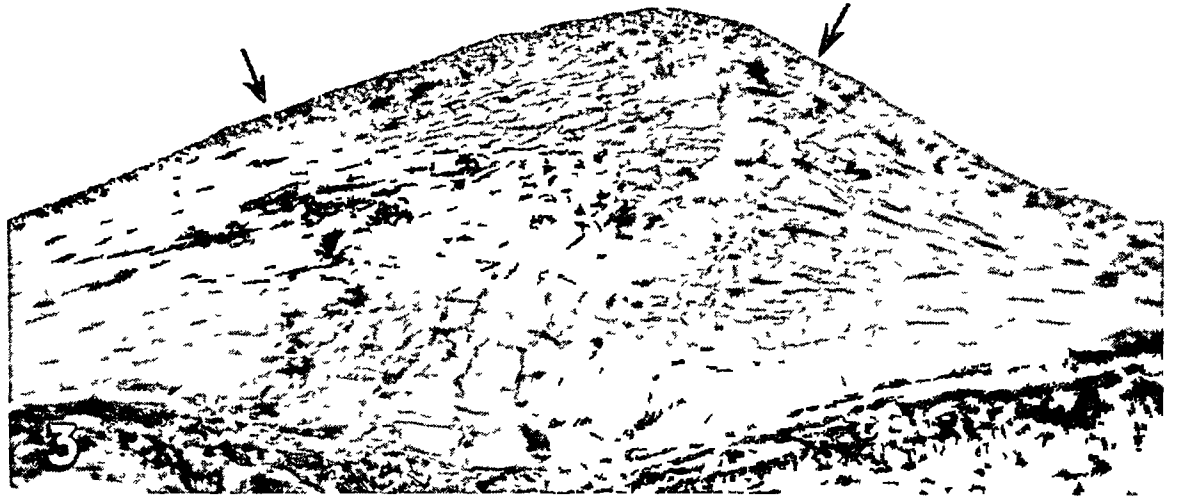
Attention is especially directed to this histologic evidence in figure 29 The nuclei in the dark stripe of the cytoplasm (figs 5, 13, 14, 20, 21, 22 and 23) are rounded, oval, dumbbell and arrow-head in shape and deeply stained In the light stripe (fig 29) they are elongated and segmented by granules A single nucleus may have many deformations, dependent on the way it straddles either a dark and a light stripe or parts of each stripe of the cytoplasm These differential deformations of the nuclei are related to the spatial distribution of the active cytoplasm of smooth muscle into dark and light stripes of condensation compression and rarefaction tension, respectively The nuclei appear to be passive and are deformed by the active cytoplasmic pressure waves This agrees with the observations of Lewis and Lewis,<sup>2</sup> who stated (p 72) "Within the nucleus, nucleolus or the mitochondria there was no change which could be considered a causal factor of the contraction or relaxation"

The spatial distribution of the cytoplasmic colloid in contracting smooth muscle under the influence of standing pressure waves is depicted in figures 9, 10, 11 and 12 The transformation of a resting medium (fig 15) into a traveling (fig 16) and into a standing wave system (fig 17) is clearly seen in the rubber tube experiment The state of resting muscle of the inner muscle coat (fig 18) is contrasted with the histologic changes produced by an overlapping traveling (fig 19) and a standing system of waves (fig 20) In the latter system the component parts, namely (1) condensation and (2) rarefaction of the frozen longitudinal pressure waves, are clearly evident whereas in the former the histologic picture is blurred by the overlapping of the elements of the wave system The contrasting pictures of the structural changes during smooth muscle contraction in the longitudinal (fig 6) and the cross section (fig 7) are clearly evident The zones of condensation and rarefaction are striking in the cross section (fig 7) of the inner smooth muscle layer



## EXPLANATION OF FIGURES 1, 2, 3 AND 4

Parts of the cross sections of the muscle wall of the large intestine of the guinea pig. Figure 1 shows resting inner smooth muscle layer,  $\times 154$ . The active inner smooth muscle layer (figs 2, 3 and 4,  $\times 38.5$ , 23 and 423, respectively), with regions of lateral expansion marked with arrows, has dark and light stripes of the cytoplasm. In the dark stripe the nuclei and cytoplasm are condensed by pressure. In the light stripe the nuclei and cytoplasm are stretched by longitudinal tension. The orientation of the dark and light stripes is better observed in the longitudinal sections of the outer muscle layer (figs 27, 28 and 29) than in the transverse sections of the intestine, which cut the inner, closely wound spiral muscle layer at a tangent in various degrees.



Figures 1, 2, 3 and 4

## EXPLANATION OF FIGURES 5, 6 AND 7

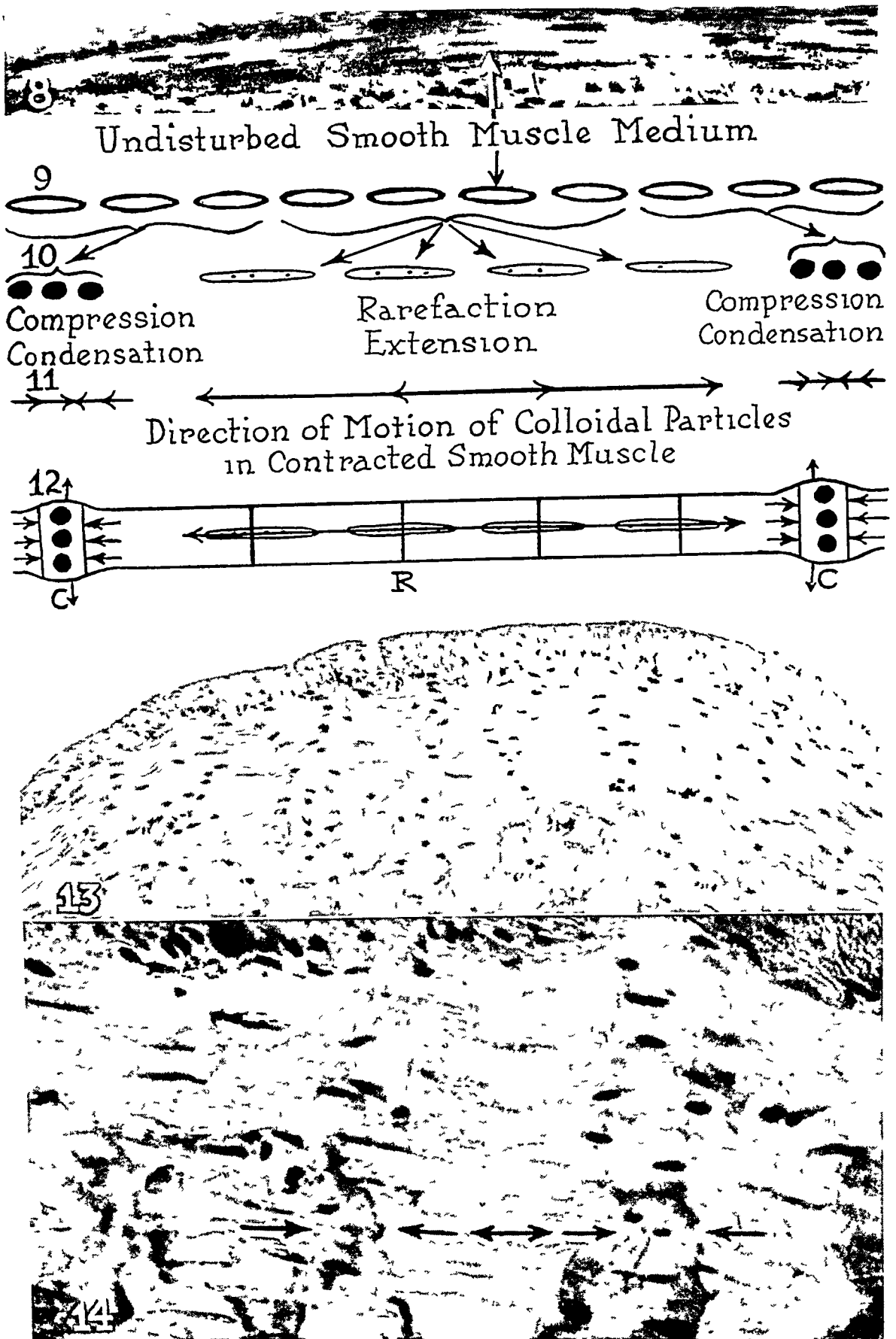
Parts of cross and longitudinal sections of the muscle wall of the large intestine of the guinea pig. Figure 5 shows the lateral expansions of the dark stripe marked by arrows. These dark stripe zones are also designated as contraction nodes with dark condensed nuclei. The lightly stained and rarefied internodal regions under stretch have elongated granular nuclei and fibrillated cytoplasm. The various deformations of the nuclei are clearly evident in both figure 5 ( $\times 423$ ) and figure 6 ( $\times 423$ ). Figure 7 shows a longitudinal section of the large intestine of the guinea pig,  $\times 423$ . The outer layer to the left is relatively at rest and cut in longitudinal section. The larger region to the right is the inner muscle layer cut in transverse section. The dark cytoplasm and nuclei are in transverse section through the condensed contraction node. The lighter regions are in transverse sections through the rarefied internodes of the inner muscle layer of the large intestine.



Figures 5, 6 and 7

## EXPLANATION OF FIGURES 8, 9, 10, 11, 12, 13 AND 14

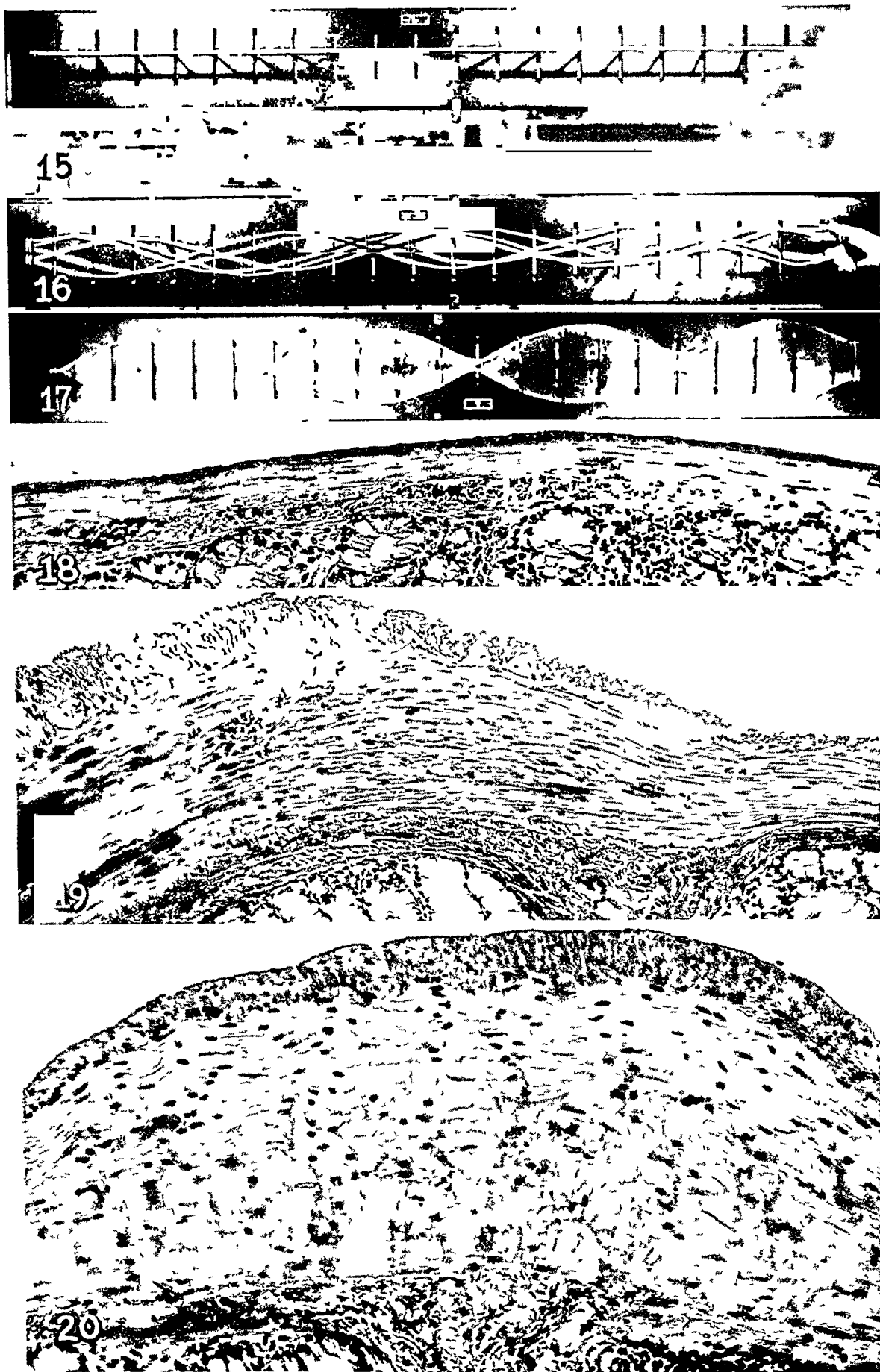
Parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figure 8 shows resting inner smooth muscle layer,  $\times 231$ . Figures 9, 10, 11 and 12 are diagrams of the changes in shape of the particles of the muscle colloid from the resting condition to that of active contraction under the influence of longitudinal pressure waves inseparably associated with the colloidal chemical changes during muscle contraction. The zones of condensation and rarefaction are the parts of each longitudinal wave of compression within the muscle. Figures 13 and 14 ( $\times 231$ ,  $\times 538.5$ ) represent transverse sections through parts of the muscle coat of the large intestine showing the spatial distribution and deformation of the cytoplasm and nuclei into longitudinal pressure waves with a dark stripe of condensation and a light one of rarefaction.



Figures 8, 9, 10, 11, 12, 13 and 14

## EXPLANATION OF FIGURES 15, 16, 17, 18, 19 AND 20

Figure 15 shows a wave apparatus. This demonstration model of transverse wave motion consists of a flexible rubber tube, 6 feet (183 cm) long, stretched horizontally and supported by twenty cross arms (Caley<sup>12</sup>). Each cross arm is connected to a ball bearing mounted on a long steel shaft around which the cross arms are free to oscillate in vertical planes. The rubber tube is loaded with shot and balanced by counterweights attached to the back end of each cross arm. The rubber tube as a transmission medium of vibration is at rest. Figure 16 shows the rubber tube experimentally activated by the motor on the left. The end of the tube at the right is free to oscillate. This freedom of motion produces a traveling system of waves. The traveling waves produce an overlapping of parts, with lack of cleancut definition of nodes and internodes. Figure 17 shows the rubber tube experimentally activated by the motor which is on the right. The end of the rubber tube on the left is fixed. Multiple reflections from the fixed end produce a standing system of waves by superposition and constructive interference with definitely cleancut nodes and internodes. The micropressure waves in the intestine are dominantly of the longitudinal type. Figures 18, 19 and 20 show parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figure 18 represents the resting inner smooth muscle layer ( $\times 231$ ), figure 19, a fixed traveling system of contraction waves originally free to travel but now "frozen," with overlapping of nodes and internodes in the inner smooth muscle layer of the large intestine ( $\times 231$ ), figure 20, stationary pressure waves transformed between intestinal ligatures from a traveling system of peristaltic waves, ( $\times 231$ ). A "frozen" system of waves consisting of condensed nodes and rarefied internodes are more definitely separated, oriented and fixed in space than those of the traveling system illustrated in figure 19. This is apparently an interference phenomenon which may be experimentally produced in muscle and which is comparable to that in the rubber tube, in the conversion of a traveling into a standing system of waves.

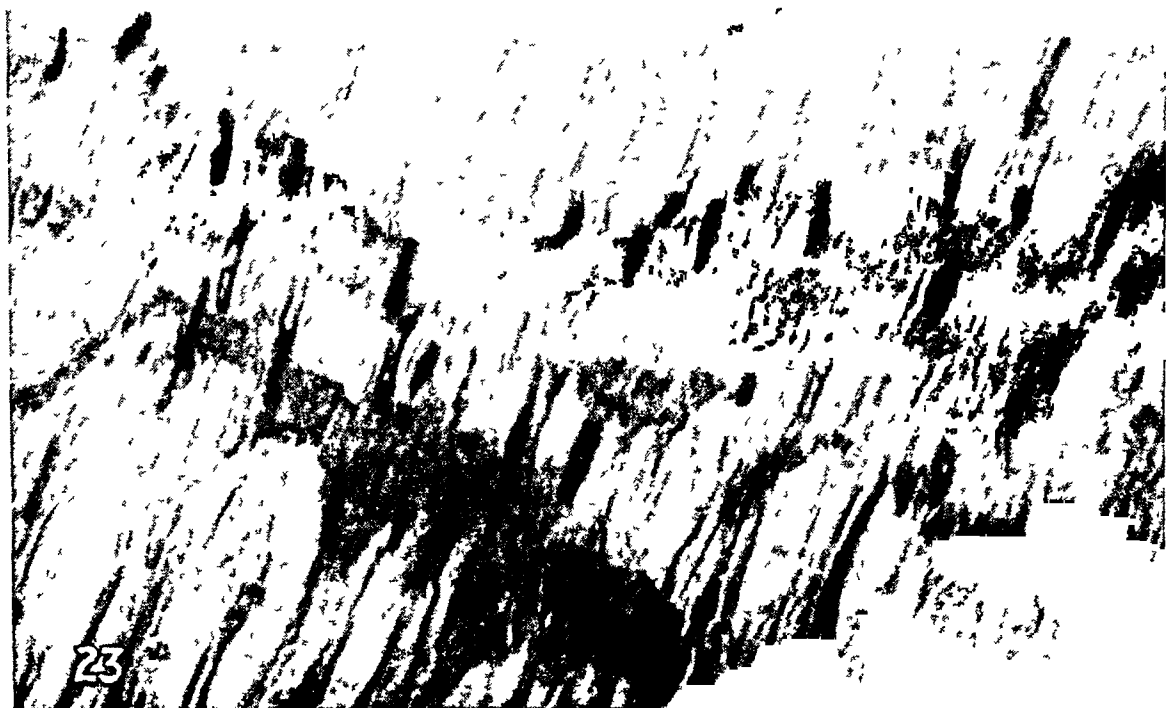
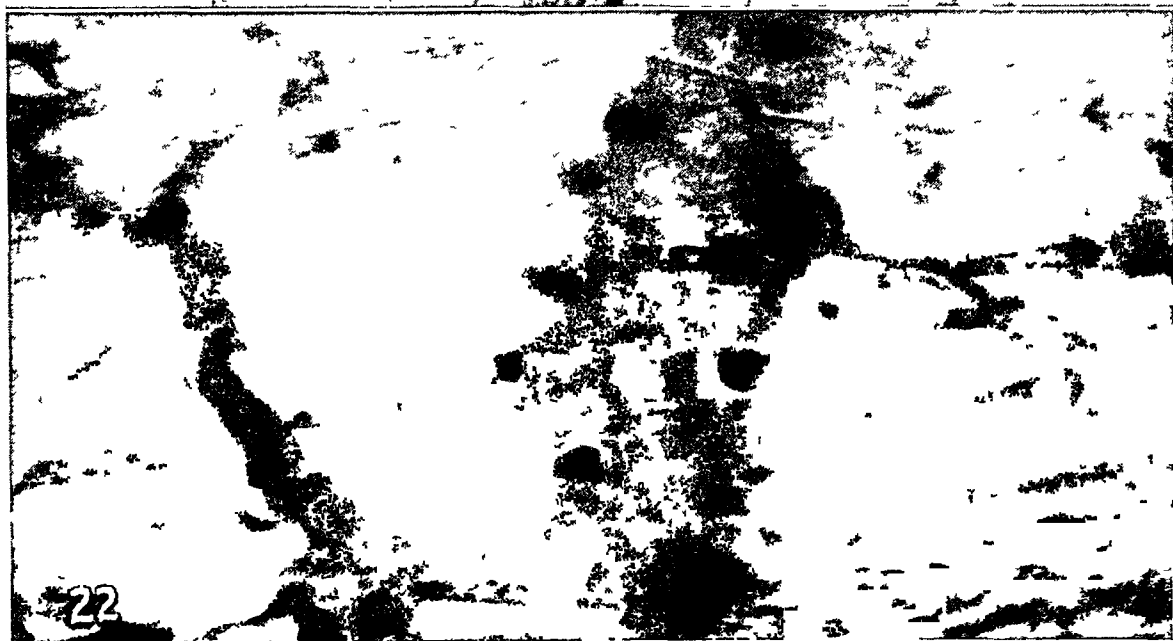
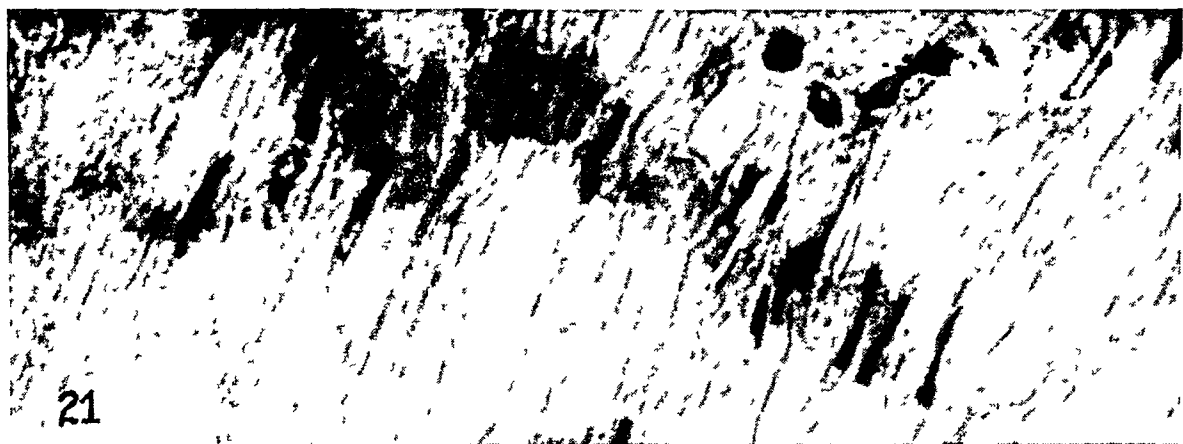


Figures 15, 16, 17, 18, 19 and 20



## EXPLANATION OF FIGURES 21, 22 AND 23

Parts of cross sections of the muscle wall of the large intestine of the guinea pig. Figures 21, 22 and 23 (all  $\times 769$ ) illustrate clearly the variable deformations of the nuclei in the zones of condensation and rarefaction. The nuclei are round, oval, tapering or compressed, and densely stained in the zone of condensation, which is the dark stripe. The zone of rarefaction is the lightly stained region. In the rarefied zone the nuclei are greatly elongated, stretched and granular. In figure 21 there are three nuclei at the right end of the dark stripe of condensation which are of dumbbell shape. In the centers of these nuclei there is a biconcave condensation, very deeply stained. The ends of these same nuclei are globular, lightly stained and granular. The various changes in shape of the nuclei are clearly evident in these various figures. The cytoplasm appears to be the region of dominant activity in smooth muscle contraction. The nuclei are relatively passively deformed by the two components of the contraction cytoplasmic wave, namely (1) the condensed contraction node and (2) the tension internode.

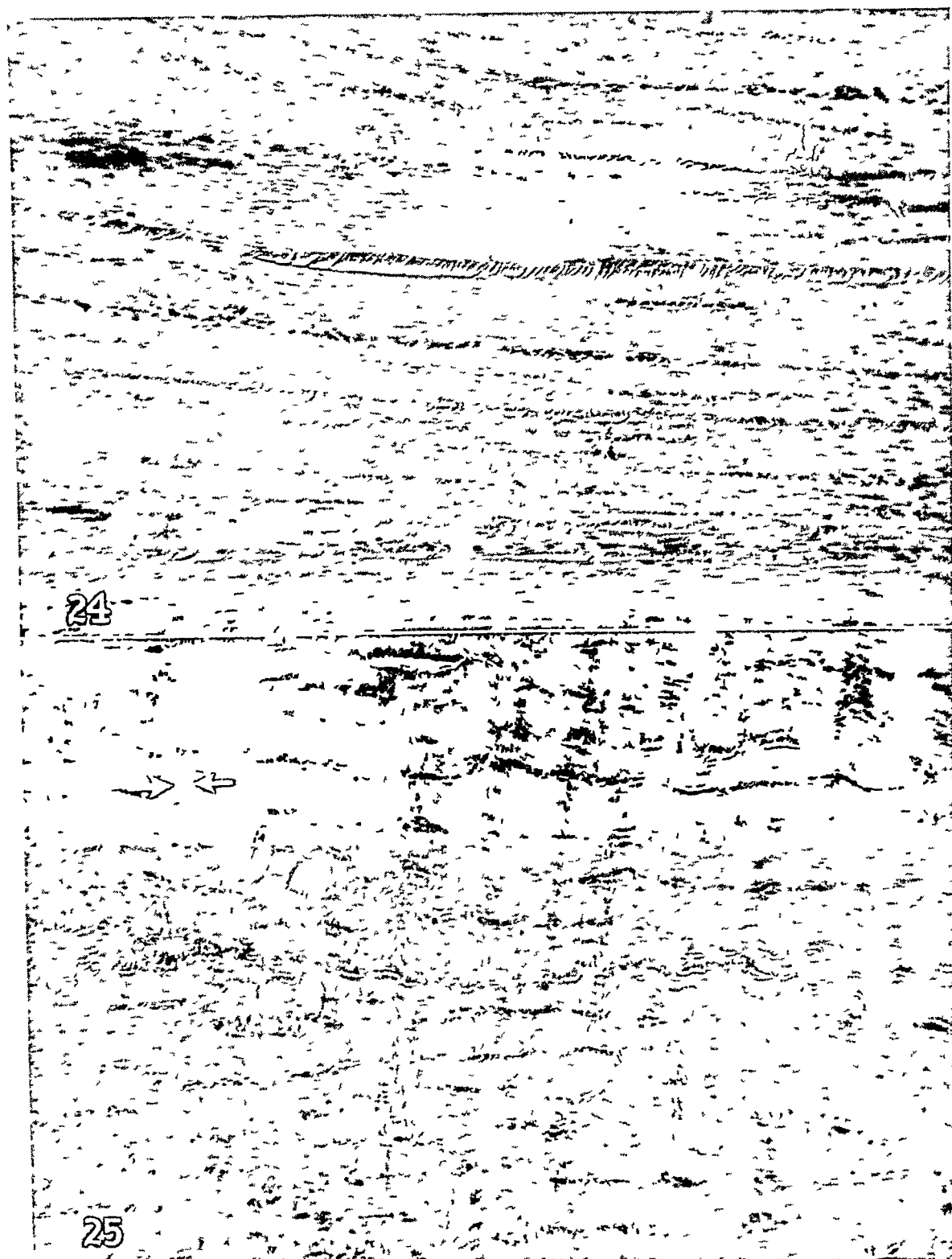


Figures 21, 22 and 23

## EXPLANATION OF FIGURES 24 AND 25

Figure 24 shows inactive muscle of the gizzard of the pigeon, ( $\times 269$ ) Experimentally it has been placed in a state of extreme inactivity by dehydration through an injection of magnesium sulfate, 2 Gm per kilogram of the body weight of the pigeon. There are no contraction waves with zones of condensation and rarefaction. Figure 25 shows a section of the gizzard of the pigeon which was placed in Ringer's solution at 39 C for ten minutes prior to fixation, ( $\times 269$ ) Zones of nodal condensation and internodal rarefaction of contraction waves are clearly evident. These microscopic waves are histologic indicators of the dynamic state of the muscle protoplasm. There appears to be a correlation between the number of these waves and the rate of the chemical reactions associated with muscle contraction and under the regulation of temperature.

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Figures 24 and 25

## EXPLANATION OF FIGURES 26, 27, 28 AND 29

Parts of longitudinal sections of the muscle wall of the large intestine of the guinea pig. Figure 26 shows resting outer smooth muscle layer ( $\times 269$ ). Figure 27, stationary pressure waves experimentally produced in the outer smooth muscle layer ( $\times 269$ ). The convexities of the outer wall overlying the contraction nodes of pressure condensation and the concavities related to the tension internodes under stretch are clearly evident. Figures 28 and 29 ( $\times 461.5$ ,  $\times 846$ ) illustrate clearly the characteristic deformations of the nuclei and cytoplasm in the zones of condensation and rarefaction of the stationary longitudinal pressure waves associated with the physicochemical changes of spastic muscle contraction. The nuclei are dark, rounded, pyknotic and condensed in the dark stripe zones of condensation. The cytoplasm is likewise clearly condensed and deeply stained in the zones of condensation, called at the present time the contraction nodes. The lightly stained tension internode has rarefied cytoplasm which is definitely fibrillated. This internodal region is under a longitudinal tension or stretch. The nuclei are elongated and granular in this internodal zone of stretch. The nuclei appear to be passively deformed by the two parts of the waves of internal compression inseparably associated with the colloidal physicochemical changes of smooth muscle contraction.



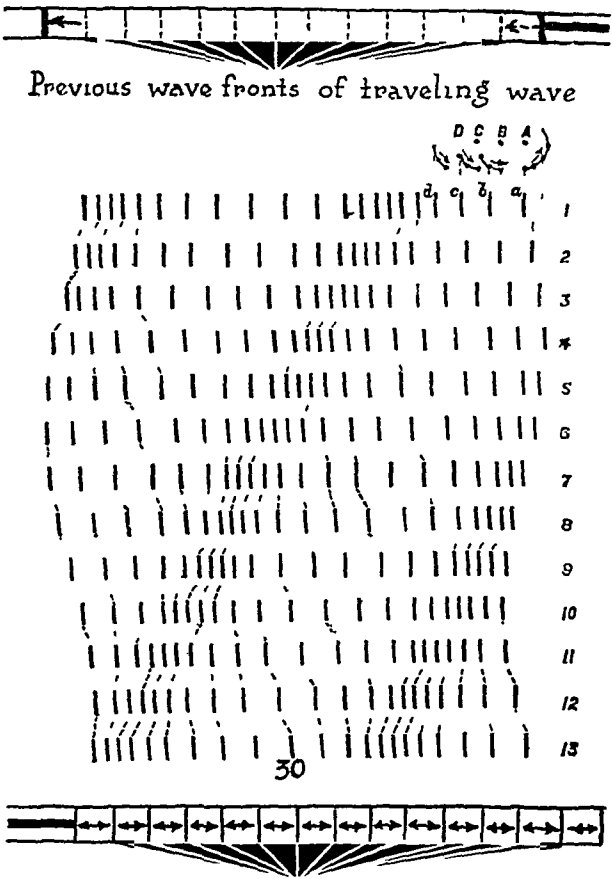
Figures 26, 27, 28 and 29

## EXPLANATION OF FIGURES 30 AND 31

Figure 30 shows progressive movements and distribution of the particles of a medium traversed by a single wave traveling from right to left. The vertical lines in each row represent plane surfaces seen edgewise, which were equidistant in the undisturbed medium. The same medium always remains between the same two planes. The successive rows 1 to 13 represent successive stages of a movement in which each of the vertical lines vibrates harmonically a specific distance to right and left of a fixed point. Each line of a row from above downward and from right to left is a little behind the line to the right of it in its movement. The mean positions of the lines are equidistant, but the lines themselves are at any one instance of time closer in some places and farther apart in others. These conditions are found farther to the left at each successive stage of the motion. If any one line is traced, by the dotted lines, from one row to another, it will be seen that when movement is to the left, i. e., in the same direction as the traveling waves of condensation and rarefaction, the line is nearest to its neighbors. When the line is moving fastest to the right it is in the most rarefied region.

Figure 31 is a diagram of movements in successive periods of time in the same medium traversed by stationary undulations. The vertical lines in rows 1 to 13 represent plane surfaces seen edgewise. These are equidistant in the undisturbed medium. The same medium is always between the same two vertical lines. The lines vibrate harmonically about their mean positions, those at the antinodes (A) have the greatest amplitude of vibration, and minimum pressure and temperature, those at the nodes (N) have zero amplitude, and maximum pressure and temperature. They all cross their mean positions together, but those on opposite sides of the same node are moving in opposite directions. In successive lines it is apparent that the medium shuttles backward and forward between relatively fixed planes, the nodes.

The fundamental spatial and temporal differences in the distribution of the particles of the medium when the latter is traversed by either a progressive traveling or a stationary system of waves are herein schematized. In the traveling wave all parts of the medium move the same distance but at different times. In the stationary system of waves all parts of the medium move at the same time but different distances. Differences in phase and amplitude of stationary waves are reflected in the distribution of the particles of the medium through different distances. (Diagrams modified after Catchpool. *Textbook of Sound*, London, Clive, 1917.)



Figures 30 and 31



The dark stripe region of cytoplasmic condensation has the following microscopic characteristics (1) anisotropy, (2) presence of inorganic ash (Carey and Zeit<sup>11</sup>), (3) compression of cytoplasm with close packing of colloidal particles, (4) rounding, deformation and compression of densely stained nuclei, (5) lateral expansion of cytoplasm. The light stripe region of rarefaction is characterized by (1) isotropy, (2) relative lack of ash (Carey and Zeit<sup>11</sup>), (3) rarefaction of loosely packed and lightly stained muscle colloids with fibrillation by stretch or tension, (4) stretching of granular nuclei to almost four times the length of those in the dark condensed stripe, (5) longitudinal tension of cytoplasm.

In the large intestine of the guinea pig the isolated group of the contraction waves that form a lateral expansion of the intestine seldom extend entirely around the circumference of the intestine (fig 2). In a traveling peristaltic wave the cytoplasm of the cell may be either totally or partially under contraction.

The average of five hundred measurements of the nuclei in inactive smooth muscle is 51 microns long and 9 microns wide. The average of five hundred measurements of the nuclei in the zone of condensation of spastic contraction of smooth muscle is 18 microns long and 15 microns wide. The average of five hundred measurements of the nuclei in the zone of rarefaction of spastic contraction of smooth muscle is 65 microns long and 5 microns wide. There are therefore an increase in the length and a decrease in the width of the nuclei in the zone of rarefaction or stretch, and a decrease in length and an increase in width of the nuclei in the zone of condensation or pressure of smooth muscle in spastic contraction, over the measurements of the nuclei of the inactive muscle.

The inactive smooth muscle of the pigeon's gizzard (fig 24) is contrasted with the active one (fig 25). The micropressure waves in the active gizzard muscle are an index of the raised level of the dynamic state, or of an acceleration in the chemical changes of metabolism under the influence of an elevation in the temperature. The acceleration in the rate of chemical reactions caused by the elevation in the temperature is accompanied by an increase in the number of micropressure waves, histologically evidenced by the close periodic spatial distribution of the cytoplasm into alternate condensed (dark) and rarefied (light) stripes and corresponding deformations of the nuclei.

The fundamental spatial and temporal differences in the distribution of the particles of the medium is schematized in figure 30, traversed by a traveling wave, and in figure 31, traversed by a standing or stationary system of waves. In the traveling wave all parts of the medium

11 Carey, E. J., and Zeit, W. *Proc Soc Exper Biol & Med* **41** 31, 1939

move the same distance at different times. In the stationary system of waves all parts of the medium move at the same time but different distances. Differences in phase and amplitude of stationary waves are reflected in the distribution of particles of medium through different distances.

#### COMMENT AND SUMMARY

Experimental microscopic evidence is presented of changes in the differential spatial distribution, deformation and physical, mechanical and staining qualities of the cytoplasm and nuclei during smooth muscle contraction. Stationary intestinal waves, experimentally transformed from traveling waves, are similar to those of inanimate matter in association with phase differences of stationary transverse or longitudinal pressure waves, such as sound or supersonic waves (Carey<sup>12</sup>). The number of micropressure waves associated with the colloidal chemical changes of active protoplasm confined in a relatively constant volume appears to be an index of the dynamic state, or metabolic activity of smooth muscle during contraction.

The variable reversible rates of the chemical reactions in smooth muscle during rest and motion appear to be associated with variable wavelengths of the micropressure waves. The increased momentum of smooth muscle motion has a complementary group of waves of micropressure. The wavelength inversely corresponds to muscle momentum. When there is a greater degree of muscle momentum and frequency of colloidal chemical change, there is a corresponding shortening of the wavelength, with a finer cytoplasmic grating of dark and light stripes.

In experimental stationary waves produced between two ligatures around the intestine alternate dark and light stripes occur in the smooth muscle cytoplasm. The dark stripe is the compression contraction node, the light one the tension internode of one complete contraction wave. This "frozen" structural expression of this immaterial contraction wave of change is demonstrated microscopically in this paper, for the first time, to have the physical attributes of a longitudinal wave of compression. The colloidal chemical reactions of both aerobic and anaerobic metabolism are associated with a micropressure wave-mechanics of protoplasmic activity of smooth muscle contraction. These explosive micropressure waves underlie the physical changes of protoplasmic activity. Physical confinement and degree of constructive interference determine the microscopic definition and orientation of these microwaves. The frequency of the oscillating colloidal particles that radiate the waves is associated with the rates of the physical and chemical changes of the active cytoplasm. The size of the colloidal

particles that determines the frequency varies with the chemical change. The site of active contraction is the cytoplasmic micropressure waves. The nuclei are relatively passive and undergo deformation by the components of the microscopic waves of longitudinal pressure.

According to the great French physicist Louis de Broglie,<sup>13</sup> it seems to be definitely established that both matter and light have two aspects. Each may be regarded either as a wave or as a substantive corpuscle. Both matter and radiation, therefore, have a dual nature.

The conclusion is made in this paper that the cytoplasmic living matter of smooth muscle is not only composed of colloidal particles but that these in action are distributed in a manner that structurally expresses underlying associated micropressure waves. The living matter of the cytoplasm of smooth muscle, therefore, seems to have two aspects, namely (1) colloidal particle and (2) the associated micropressure wave.

Mr. Leo Massopust assisted with the photomicrographs.

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<sup>13</sup> de Broglie, L. *Matter and Light*, New York, W. W. Norton & Company, Inc., 1939, p. 48.

# CAPILLARY RUPTURE WITH INTIMAL HEMORRHAGE IN THE CAUSATION OF CEREBRAL VASCULAR LESIONS

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OTTAWA, CANADA

Capillary rupture with intimal hemorrhage in relation to the precipitation of coronary thrombi has been described in detail elsewhere,<sup>1</sup> the observations reported there, and in part the conclusions, have been confirmed and elaborated on by Wartman,<sup>2</sup> by Winternitz and his co-workers<sup>3</sup> and by others. It has been shown that intimal hemorrhages result from the rupture of capillaries derived from the arterial lumens, not from the backflow of blood through intimal defects as was previously thought. Because intimal hemorrhages are a common finding at the sites of precipitation of coronary thrombi, it appears fairly certain that the two lesions are cause and effect. Recently I have suggested that pulmonary thrombi may sometimes be precipitated by similar intimal hemorrhages.<sup>4</sup>

The purpose of the present paper is to describe intimal hemorrhages due to capillary ruptures in sclerotic cerebral arteries and to discuss the relation of these hemorrhages to certain cerebral vascular lesions, namely, arteriospasm, thrombosis and cerebral hemorrhage.

## MATERIAL AND METHOD

Most of the material for this study was obtained at autopsies on 6 patients who had shown clinical signs of cerebral thrombosis. When the thrombus was identified on gross examination, the affected segment of the artery was embedded in one or more blocks and sectioned serially at intervals of about 100 microns. When the thrombus was not evident on gross examination, the entire involved artery was cut into short segments, and these were embedded in bundles and sectioned serially at intervals of about 100 microns. When lesions of interest were noted on microscopic examination, the intervening sections were mounted and stained. In case 1 all of the cerebral arteries and their main branches were studied by serial section, the "bundle" method being used. Most of the sections were stained with hematoxylin and eosin, but occasionally Perle's stain and Masson's trichrome light green stain were used.

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From the Department of Pathology of the Ottawa Civic Hospital

1 Paterson, J C Arch Path **22** 313, 1936, **25** 474, 1938, J A M A **112** 895, 1939

2 Wartman, W B Am Heart J **15** 459, 1938

3 Winternitz, M C, Thomas, R M, and LeCompte, P M The Biology of Arteriosclerosis, Springfield, Ill, Charles C Thomas, Publisher, 1938

4 Paterson, J C Am Heart J **18** 451, 1939

The remainder of the material was obtained from the department of neuropathology of the University of Toronto with the aid of Prof Eric Linell. It consisted of 3 hemorrhagic lesions of the cerebral arteries, each from a different case. Two were from the basilar artery, and the third was from the left middle cerebral artery. The latter was studied by partial serial section.

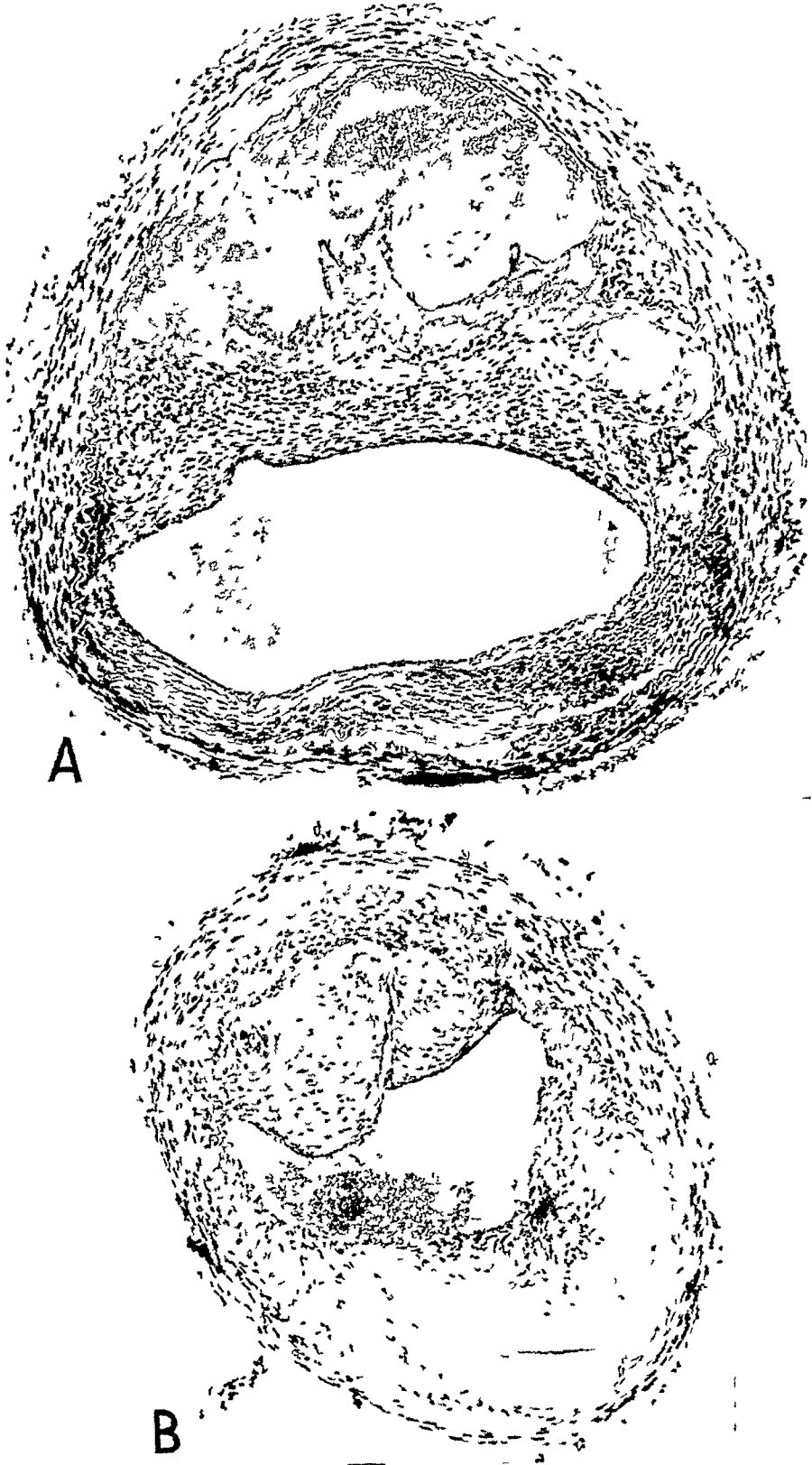
#### OBSERVATIONS

Intimal hemorrhages of sclerotic cerebral arteries have been found both with and without thrombosis of the adjacent arterial lumens. The hemorrhages were similar in all respects to those described in sclerotic coronary arteries. Serial section through several of them showed the hemorrhage to be confined to the deeper layers of the thickened intima, the more superficial intimal layers and the endothelium being intact (fig 1 *A*). The intimal hemorrhages were observed only in areas of atheromatous degeneration. In many instances small capillaries lay in proximity to the extravasated blood and to the lumen of the artery. Serial section showed these capillaries to arise from the arterial lumen (fig 1 *B*). Frequently, in individual cases, intimal hemorrhages were multiple in the cerebral arteries, and sometimes similar hemorrhages were found as well in other arteries of the musculoelastic type. For example, in 1 case there was an intimal hemorrhage at the site of thrombosis in a middle cerebral artery, 11 other intimal hemorrhages in various parts of the cerebral circulation, a large intimal hemorrhage resembling a localized dissecting aneurysm in the abdominal aorta and an intimal hemorrhage of the right coronary artery which produced stenosis of the lumen.

Certain possible sequelae of intimal hemorrhages in sclerotic cerebral arteries were suggested in the series. In the first place, 1 case presented an interesting association of symptoms resembling cerebral arteriospastic attacks, and at autopsy multiple intimal hemorrhages of varying ages were observed in the cerebral arteries. A summary of the history of this case follows.

CASE 1—A 63 year old, obese woman, known to have had diabetes mellitus and hypertension for three years, complained of frequent headaches, fatigue, defective memory and numerous falls over the same period of time. Most of the falls occurred when she tripped over some object, but she had also fallen for no apparent reason when walking on level ground. She had fallen downstairs three times. She was admitted to the hospital with signs and symptoms of cerebral arterial thrombosis. The blood sugar on admission was 314 mg per hundred cubic centimeters, and the blood pressure was 178 systolic and 118 diastolic. Forty-five days later she died with signs of bronchopneumonia.

The principal abnormalities noted at autopsy were atrophy of the pancreas with fibrosis of the islets of Langerhans, marked atherosclerosis of the aorta, coronary and cerebral arteries, multiple intimal hemorrhages of the aorta, coronary and cerebral arteries, thrombosis of a middle cerebral artery, softening of the right parietal lobe of the brain, thrombosis of the femoral veins and pulmonary embolism.



#### EXPLANATION OF FIGURE 1

*A*, photomicrograph of one of the intimal hemorrhages in case 1. The hemorrhage lies close to the media in the outer zone of an atheromatous focus. Hematoxylin and eosin,  $\times 45$ .

*B*, photomicrograph of one of the main cerebral arteries in case 1 showing a capillary arising from the arterial lumen and traversing the thickened intima. Hematoxylin and eosin,  $\times 45$ .

Each of the coronary arteries showed numerous points of stenosis due to the presence of atheromatous plaques. The right coronary artery, at a point 6 cm from its origin, was almost completely stenosed by a plaque into which massive hemorrhage had occurred. The aorta was also markedly atherosclerotic and showed numerous points of intimal erosion. Underlying an erosion in the abdominal aorta was a large intramural hematoma, measuring 4 cm in diameter. The main branches of the cerebral arteries showed gross evidence of athero-

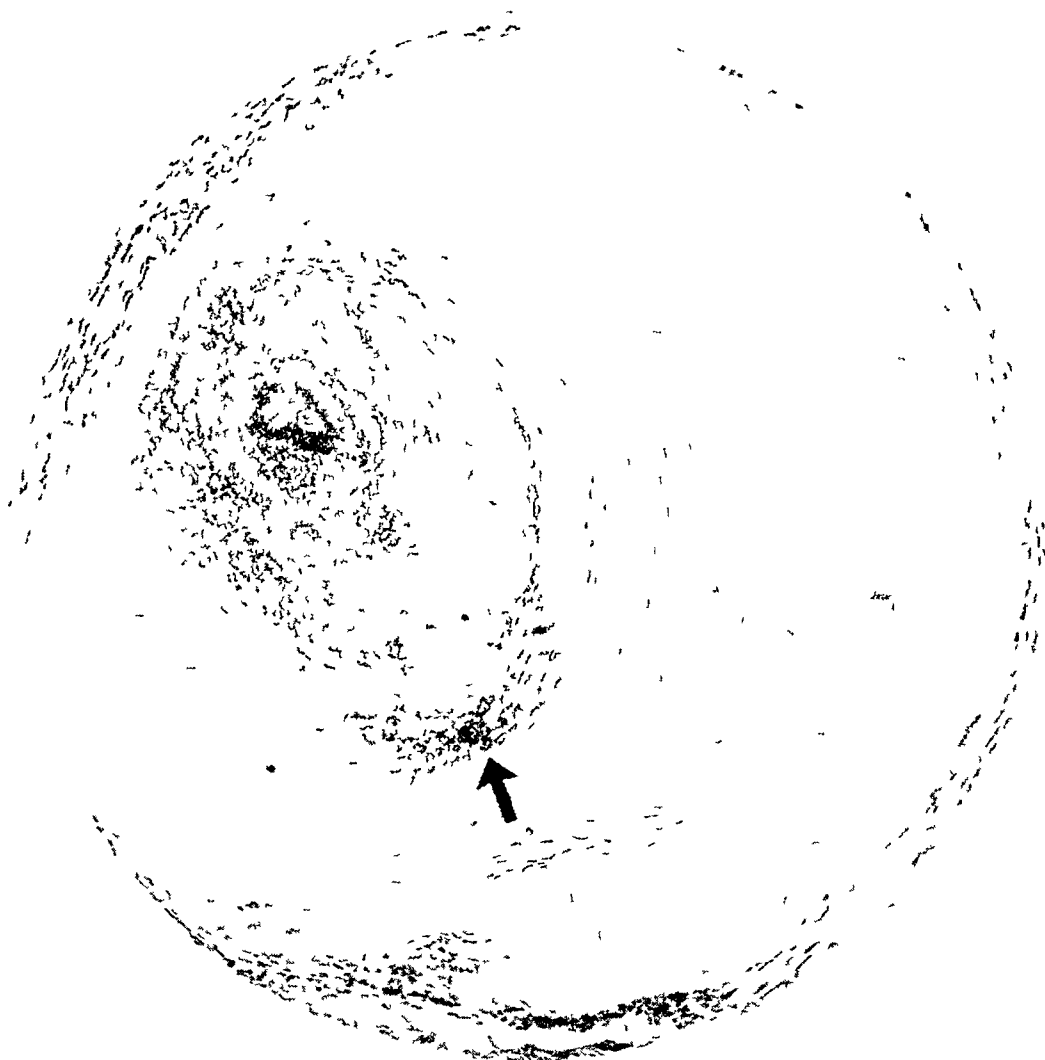


Fig 2—A low power photomicrograph of a thrombosed cerebral artery. A small intimal hemorrhage (shown by the arrow) lies close to the oldest part of the thrombus. Hematoxylin and eosin,  $\times 20$ .

sclerosis with numerous points of stenosis. Projecting from the outer surface of the left posterior cerebral artery was a reddish brown rounded nipple-like mass, measuring 3 mm in diameter. Two similar hemorrhagic lesions were noted on the outer surface of the right posterior cerebral artery. These 3 hemorrhages were embedded separately and sectioned serially. The remaining portions of the principal cerebral arteries were cut into short segments, embedded in bundles, and sectioned serially at intervals of 200 microns.

Microscopically, 12 intimal hemorrhages, including the 3 noted on gross examination, were found in various parts of the principal cerebral arteries. Each hemorrhage had occurred into an area of atheromatous degeneration. Occasionally the lumen appeared to be stenosed by the hemorrhage. Many of the intimal hemorrhages lay in the outer zones of the thickened intima, in contact with the media (fig 1A). The hemorrhages varied in age, some consisted of intact red cells, while in others the red cells had disintegrated, and stainable iron was present. One artery with an outside diameter of 2.5 mm showed almost complete stenosis of the lumen by atherosclerotic thickening of the intima, recent hemorrhage into the outer zone of the intima and complete occlusion of the lumen by thrombus material of fairly recent formation. Capillaries lay in proximity to several of the intimal hemorrhages, and in 1 instance serial section showed one of these capillaries to arise from the lumen of the artery. Sections through the intimal hemorrhage in the right coronary artery showed marked stenosis of the lumen by an atheromatous plaque in which there was a large amount of hemorrhage, consisting of disintegrating red cells and yellowish pigment. The hemorrhage was so massive that it had obviously compressed the already stenosed coronary lumen. Sections through the intramural hematoma of the abdominal aorta showed the hemorrhage to be confined to a large intimal aneurysm. Numbers of capillaries lay between it and the endothelial lining.

Six cases of cerebral thrombosis have been studied to date. The thrombosed segments of the arteries were sectioned serially throughout their length. Intimal hemorrhage was found at the point of thrombus precipitation in 4 of the 6 cases. When parts of a single thrombus varied in age (as determined by the amount of organization), the oldest part was attached to the arterial wall close to an intimal hemorrhage (fig 2). Both the thrombus and the intimal hemorrhages were of recent origin in 3 of the 4 cases, while in 1 case the thrombus was organized and the intimal hemorrhage largely converted into pigment. In this case there was also an organized thrombus with an old intimal hemorrhage in the right coronary artery, as well as a number of fresh intimal hemorrhages in both branches of the left coronary artery. Each of the cerebral thrombi which were associated with intimal hemorrhages had formed at a point of stenosis of the lumen of the artery. Capillaries lay in proximity to the intimal hemorrhages and to the arterial lumens in several cases.

Finally, a case has been observed in which an intimal hemorrhage had ruptured through the medial coat of the artery and leaked into and through the adventitial fibers (fig 3). A summary of the history and the observations at autopsy in this case follows.

CASE 2—A 72 year old man gave a history of senile mental deficiency, poor memory and inability to move the left arm for many years. Just before admission swelling of the feet and ankles developed, with breathlessness on the slightest exertion. The blood pressure on admission was 150 systolic and 90 diastolic, but later it rose to 184 systolic and 100 diastolic. Signs of bilateral bronchopneumonia developed and the patient died about two weeks after admission.



The principal observations at autopsy were confined to the brain and meninges. The dura mater covering the inner surface of the convexity of the left cerebral hemisphere was thickened up to 2 mm by a deposit of orange-brown old blood pigment covered by a smooth shining membrane. The large arteries at the base of the brain showed marked atherosclerosis. The left middle cerebral artery at a point just beneath the tip of the temporal lobe was markedly sclerotic over a distance of about 1 cm, and over most of this area the wall of the vessel was

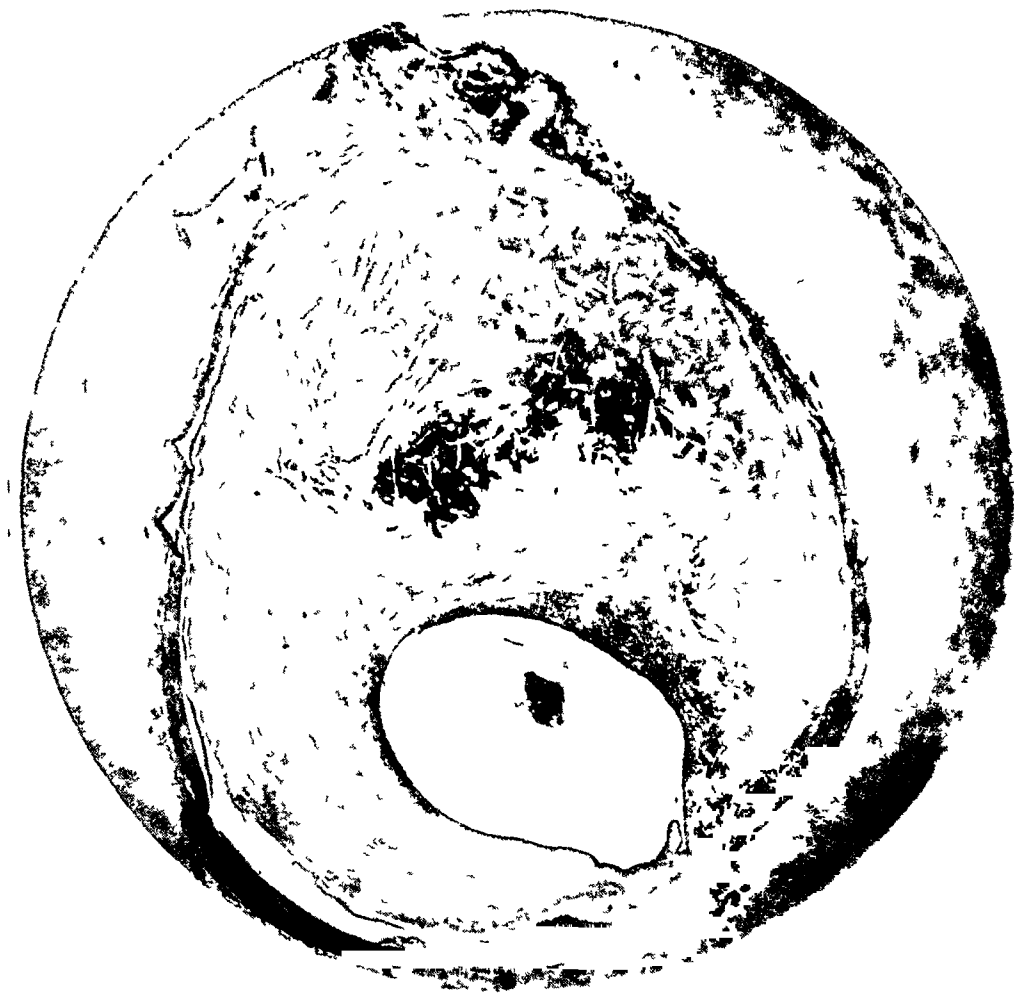


Fig. 3—Photomicrograph of a cerebral artery in case 2 in which a large intimal hematoma has broken through the media and involves the adventitial coat. Hematoxylin and eosin,  $\times 18$ .

distended. In one segment of the sclerotic patch there was a nipple-like projection of the adventitial coat, the outer layers of which were stained with old blood pigment. Examination of the brain tissue revealed bilateral internal hydrocephalus of the lateral ventricles, and a cystic defect, measuring 1 cm in diameter, of the white matter overlying the roof of the posterior horn of the right lateral ventricle. One half of the nipple-like projection of the left middle cerebral artery was sectioned serially at intervals of 8 microns.

Microscopically, the projecting mass on the left middle cerebral artery was made up of a large hematoma consisting of both fresh and old hemorrhage. The hemorrhage lay for the most part in the outer half of a large atheromatous plaque of the intima, but in some of the sections it had broken through the media and lay among the adventitial fibers (fig 3). A thick layer of dense fibrous tissue separated the intimal hemorrhage from the lumen of the artery. No intimal capillaries were noted in the sections studied.

#### COMMENT

Intimal hemorrhages in sclerotic cerebral arteries are identical in structure with those described previously in atherosclerotic plaques of human coronary arteries. They occur only in atheromatous foci, and they are intimately related to capillaries which arise from the arterial lumens. It is generally admitted, now, that intimal hemorrhages of the coronary arteries are intrinsic lesions resulting from the rupture of capillaries which are derived from the lumens of the main arteries. It may be assumed that intimal hemorrhages of the cerebral arteries are produced in a similar manner. This assumption is supported by the fact that coronary and cerebral intimal hemorrhages are not infrequently found in the same patient, a fact which suggests a common etiologic agent.

Likewise, the precipitating factor of thrombosis in sclerotic cerebral arteries appears to be the same as that in thrombosis of the coronary arteries. I have described elsewhere<sup>1</sup> the common finding of intimal hemorrhages at the points of precipitation of coronary thrombi, and this has been confirmed by others.<sup>5</sup> To date, 47 cases of coronary thrombosis have been studied by fairly exhaustive methods, including serial sections in many instances, and in 41 cases intimal hemorrhage was found at the site of precipitation of the thrombus. The common association of intimal hemorrhages and coronary thrombi in a series as large as this and the confirmatory evidence of others eliminate the possibility of this association being coincidence. Because intimal hemorrhage often occurs without thrombosis of the adjacent arterial lumen, such hemorrhages cannot be regarded as resulting from the presence of the thrombi. One is forced to the conclusion, therefore, that most coronary thrombi are precipitated either by the intimal hemorrhages proper or by other lesions that result from the rupture of intimal capillaries. The same conclusion must be reached in regard to thrombi in cerebral arteries. Although my series is small the intimal hemorrhages found at the sites of precipitation of 4 of 6 cerebral thrombi were identical in all respects with those observed in coronary arteries.

A certain amount of evidence has been collected to show that other cerebral vascular lesions besides thrombosis may be related to intimal

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<sup>5</sup> Finkelstein, L., and Horn, H. Personal communication to the author.

hemorrhages Of particular interest is their relation to arteriospastic attacks in hypertensive persons as suggested in case 1 in this series The clinical signs of cerebral arteriospastic attacks over a period of four years in this case and the postmortem observation of 12 distinct intimal hemorrhages of varying ages in the larger cerebral arteries suggest that they were related Many of the hemorrhages lay at the outer borders of atheromatous plaques in proximity to the media (fig 1 *A*), and it is reasonable to suppose that the sudden disruption of tissue by the hemorrhages may have set up transient spasms of the muscle coat The observations of others tend to confirm this hypothesis The walls of cerebral arteries are known to be supplied by vasomotor fibers, and local spasm due to local injuries or influences definitely occurs Stroking the adventitia of a pial artery with a blunt instrument at operation causes spasm of that part of the vessel<sup>6</sup> Also, injuries to arterial walls are known to cause pain Waterston<sup>7</sup> found that the contact of the point of a needle with the wall of an artery elicited sharp pain, and when the needle point was pushed into the wall a peculiar sickening pain, associated with nausea and faintness, resulted Feiling,<sup>8</sup> and Aring and Merritt<sup>9</sup> commented on the frequency with which certain prodromal symptoms occur in patients with cerebral arterial thrombosis The symptoms consist of headache, dizziness, transient weakness of one or both limbs or of one side of the face, temporary aphasia and other symptoms Sometimes slight hemiplegic weakness may appear and rapidly vanish, to be followed a few days later by severe hemiplegia It is possible that these premonitory symptoms in certain cases, including pain in the head, are due to arteriospasm from irritation by the intimal hemorrhages which precede and apparently cause cerebral thrombosis It must be admitted, however, that attempts to demonstrate nerve fibers in the actual area of intimal hemorrhage have as yet been unsuccessful

Equally hypothetic is the relation of intimal hemorrhage to cerebral arterial rupture An accidental finding in a case in my series was a large intimal hematoma of a middle cerebral artery which had broken through the thin medial layer and lay between the adventitial fibers (fig 3) It is possible that a true intracranial hemorrhage would have occurred in this case if the process had continued Cases of classic cerebral hemorrhage due to rupture of the lenticulostriate artery have not as yet been studied, but the observations of others suggest that in this vessel also the precursor of rupture may be an intimal hematoma

6 Penfield, W Personal communication to the author

7 Waterston, D *Lancet* **1** 943, 1933

8 Feiling, A *Practitioner* **133** 62, 1934

9 Aring, C D, and Merritt, H H *Arch Int Med* **56** 435, 1935

Ellis<sup>10</sup> found that the miliary aneurysms which are usually regarded as the cause of cerebral hemorrhage are not true aneurysms but are encapsulated hematomas. This important question is now being studied and will be discussed later.

From the observations in this short series of cases it appears that capillary rupture with intimal hemorrhage is intimately concerned with the mechanism of cerebral arterial thrombosis and possibly with the causation of cerebral arteriospasm and hemorrhage in certain cases. If this is true, the factors responsible for the rupture of intimal capillaries should be the immediate causes of these lesions. The factors responsible for the rupture of intimal capillaries in the coronary arteries have been described elsewhere,<sup>1</sup> and the same factors hold good for the cerebral vessels. The integrity of a capillary wall in any part of the body depends on the pressure of blood within its lumen, the strength and elasticity of its wall, and the rigidity of its supporting stroma. Because intimal capillaries arise directly from the lumen of a large artery, it is argued that they will be subjected to a greater blood pressure than those which lie at the end of a long series of arteries and arterioles. This argument is not new. Ellis<sup>10</sup> stated that "the small vessels of the basal ganglia, notoriously the site of hemorrhage, are direct branches of the cerebral arteries and hence are subjected to a blood pressure greater than vessels of similar size in other parts of the brain and possibly of the body." It is reasonable to assume that the pressure in intimal capillaries of the cerebral arteries, normally high, will be increased in cases of persistent hypertension or of temporary elevation of blood pressure from excessive exercise or emotion. In these circumstances the strain on the capillary walls will be increased, and there will be imminent danger of capillary dilatation and rupture. In the series reported here, persistent hypertension was present in 3 of the 4 cases in which intimal hemorrhage was observed at the site of precipitation of a thrombus. Aving and Merritt<sup>9</sup> investigated 96 cases of cerebral thrombosis and found that in 85 per cent the systolic pressure was in excess of 140 mm of mercury and that in 50 per cent the diastolic pressure was in excess of 100 mm of mercury.

Loss of rigidity of the tissues supporting the capillary walls appears to be equally important in the causation of capillary rupture in cerebral arteries. Each of the intimal hemorrhages in this series was found in an area of atheromatous degeneration, the softening process which is characteristic of atheroma allows the capillaries to dilate, and if the intracapillary pressure is high enough, overdilatation and rupture may occur. Theoretically, replacement of the atheromatous material by

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10 Ellis, A. G. *Publ. Jefferson M. Coll. & Hosp.* 5:1, 1915.

calific deposits should help to strengthen the supporting stroma and prevent capillary dilatation and rupture, but it is noteworthy that calcification in the cerebral arteries is rare, and none at all was seen in this series

Finally, the strength and elasticity of the walls of capillaries in cerebral arteries may be affected by a number of factors. It is known that capillary fragility increases with advancing age, vitamin deficiency or local inflammatory conditions. Each of these factors may be of importance in the production of intimal hemorrhage in certain cases

#### SUMMARY

Intimal hemorrhages in sclerotic cerebral arteries are similar in structure to those described previously in sclerotic coronary arteries. They result, not from backflow of blood through defects produced by rupture of atheromatous "abscesses," as was previously thought, but from rupture of capillaries derived from the main arterial lumens.

From the observations in this series it appears that capillary rupture with intimal hemorrhage is intimately concerned with the mechanism of cerebral arterial thrombosis and possibly, in certain cases, with the causation of cerebral arteriospasm and rupture. It is suggested that the factors responsible for the rupture of intimal capillaries in the cerebral arteries are high intracapillary pressure from hypertension, progressive atheromatous degeneration of the supporting tissues and increased capillary fragility from a variety of causes.

# EFFECTS OF PROLONGED INJECTIONS OF BOVINE ANTERIOR PITUITARY EXTRACT ON BONE AND CARTILAGE OF GUINEA PIGS

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AND

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In immature guinea pigs injections of an acid extract of the anterior lobe of the bovine pituitary for periods of from four to twenty-one days promoted the formation of cartilage and bone<sup>1</sup> The course of endochondral ossification was determined by the rate at which the proliferation of the cartilage and its subsequent replacement by bone took place<sup>2</sup> In the majority of cases the calcification and ossification of the cartilage predominated over the new formation of cartilage cells and this led to narrowing of the epiphysial disk In other instances, however, the proliferation of cartilage was more stimulated than ossification and the epiphysial lines, therefore, remained open In order to determine whether these reactions represented a temporary condition or changes of a more permanent nature, we investigated the alterations in the skeletal tissues after administration of the anterior pituitary extract for longer periods of time

## MATERIAL AND METHODS

Thirty-seven guinea pigs of both sexes, born at different seasons and with an initial weight of from 135 to 175 Gm, were used Each of eighteen guinea pigs received a daily intraperitoneal injection of 1 cc of a freshly prepared acid extract of the anterior lobe of the bovine pituitary for from one to six months, while 19 guinea pigs served as controls

Of a total of 18 treated guinea pigs, 5 received injections for one month, 5 for two months, 2 for three months, 2 for four months and 1 for six months Two additional guinea pigs which had been given injections for three months and another which had been treated for four months were allowed to survive for an additional period of four months, during which no injections were given

As to the technical procedure of removing the bones and preparing the specimens, we refer to our previous reports<sup>2</sup>

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1 Silberberg, M Proc Soc Exper Biol & Med **32** 1423, 1935

2 Silberberg, M, and Silberberg, R Arch Path **26** 1208, 1938

## GENERAL OBSERVATIONS

The mean weights of the guinea pigs are given in table 1

At early stages the gain in weight in the treated guinea pigs was less than in the control animals. At later stages, in the majority of the treated animals, even in those which had, for a while, shown a dwarfed appearance, a considerable gain in

TABLE 1—*Mean Weights and Deviations from the Means of Normal and Treated Guinea Pigs*

	Total Number	Initial Weight, Gm	Weights After					
			1 Mo	2 Mo	3 Mo	4 Mo	5 Mo	6 Mo
Normal animals	19	(19 animals)	(19 animals)	(15 animals)	(11 animals)	(8 animals)	(6 animals)	(1 animal)
		140 { +15 -10	242 { +63 -57	311 { +89 -96	431 { +129 -106	520 { +40 -80	609 { +116 -49	640 { +0 -0
Treated animals	18	(18 animals)	(18 animals)	(13 animals)	(8 animals)	(1 animal)	(1 animal)	(1 animal)
		149 { +26 -14	194 { +36 -64	279 { +101 -139	352 { +108 -122	438 { +17 -103	585 { +0 -0	615 { +0 -0

TABLE 2—*Individual Weights of Two Pairs of Guinea Pigs Which Had Been Given Injections for Two and Three Months, One Pair Having Been Allowed to Survive After Discontinuation of the Injections*

Initial weight, Gm	Guinea Pig 170/114			Guinea Pig 171/114			Guinea Pig 43			Guinea Pig 14		
	185			180			140			145		
1 week*	175	175	165	175	175	175	150	140	160	145	125	140
2 weeks	180	180	185	180	185	180	155	160	175	140	155	140
3 weeks	190	210	200	180	200	195	160	145	150	130	125	145
4 weeks	205	230	215	205	225	210	170	175	160	140	140	135
5 weeks	220	230	230	220	215	255	155	155	160	125	130	135
6 weeks	195	235	235	230	260	265	180	170	180	145	140	160
7 weeks	250	235	265	290	290	325	205	210	200	165	170	175
8 weeks	260	265	275	320	345	380	205	190	200	180	165	180
9 weeks							190	160	180	190	175	190
10 weeks							175	180	190	195	200	215
11 weeks							185	155	195	205	175	220
12 weeks							160	170	160	205	210	215
13 weeks							200	195	230	230	205	230
Injections discontinued												
14 weeks								315			340	
15 weeks								330			350	
16 weeks								350			340	
17 weeks								370			350	
18 weeks								420			405	
19 weeks								385			370	
20 weeks								400			390	
Onset of pregnancies												

\* The bold face numbers mark either a lack of gain or a loss in weight

weight and comparatively fast growth were seen, after three months the animals could hardly be distinguished from normal controls. This agrees with the finding of Loeb<sup>3</sup> that a marked decrease in hyperactivity of the thyroid gland takes place step by step in the course of prolonged injections of anterior pituitary extract.

The individual weights of two pairs of guinea pigs are given in table 2. Each pair had been treated at the same time and with the same dose of the same extract,

3 (a) Loeb, L., and Basset, R. B. *Proc Soc Exper Biol & Med* **26** 860, 1929. (b) Loeb, L., and Friedman, H. *ibid* **29** 172, 1931.

nevertheless, the weight curves differed, one rising steadily after an initial period of arrest of weight, and the other showing a second period of cessation of weight increase, following a period of growth. The weights of these animals seem of interest with reference to the question whether periods of cessation in gain or periods of loss of weight may be correlated with so-called lines of arrested growth in bone.

In table 2 we have marked periods of lack of gain in weight or loss in weight with asterisks.

Measurements of the length of the tibia and femur were taken by means of a caliper. In regard to these we found, however, even in normal animals, considerable variations, amounting to as much as 10 per cent and more, between different guinea pigs. For instance, with normal guinea pigs weighing 180 to 190 Gm. it was found that the length of the tibia was 3.5 cm. in some and only 3.1 cm. in others. Similar variations were found in the treated animals. In view of these variations, and because we did not use standardized strains of animals in our experiments, we did not think it feasible to draw definite conclusions from the measurements of the bones. Instead, we relied on the histologic study of these tissues.

#### MICROSCOPIC OBSERVATIONS

As to the normal microscopic structure of the bone and cartilage of the guinea pig at different ages, we refer to former publications.<sup>1</sup>

After one month's treatment with the anterior pituitary extract the cartilage and bone at the upper epiphyseal zone of the tibia were seen to react in one of two ways:

1. In 3 of 5 animals which had lost or which had not gained weight the zone of endochondral ossification was distinctly narrowed. The intercartilaginous ground substance was acidophilic, sclerosed, and resembled piecoseous substance. Instead of ten columnar cells as observed ordinarily, only five to six, or even fewer, cells were counted in the epiphyseal cartilage row, instead of the normal number of four hypertrophic cells in one row, only two cells or a single one was found. Sometimes whole rows of cartilage cells had undergone retrogression, such circumscribed areas were filled in with wedge-like osseous plugs. In general, an intensified calcification of the cells of the hypertrophic layer was associated with or followed by rapid ossification, either by breakdown of cartilage cells following ingrowth of capillaries into the cartilage and subsequent replacement by bone or, here and there, by direct conversion of hypertrophic cartilage cells into osteocytes. In the subepiphyseal layer the trabeculae were numerous and thickened, and their main axes were arranged in a transverse as well as in a longitudinal direction, if, however, fibrosis of the marrow was found, the trabeculae were thinned out, contained much calcium and were arranged in a longitudinal direction or were destroyed by the proliferating connective tissue of the bone marrow. In the chondrophyte the intercartilaginous matrix was likewise sclerosed, some cartilage cells were hyperplastic, and hypertrophic incubator capsules appeared, which in places underwent retrogression. The cartilage of the joint was thickened, the cartilage cells, particularly those of the transitional and pressure zones, proliferated by way of amitosis, they took on a perpendicular arrangement, and four and more cells were surrounded by a common capsule. Some of these cells underwent hypertrophy followed by karyorrhexis and karyolysis. The bony border lamella in some areas was corroded by capillaries.

<sup>1</sup> Silberberg, M., and Silberberg, R. *Am. J. Path.* (a) **15**: 547, 1939, (b) **15**: 55, 1939, footnote 2.



advancing from the bone marrow of the epiphysis, however, calcification and ossification also progressed. In ribs and vertebrae the state of cartilage and bone was similar to that in the long bones.

2 In 2 of 5 guinea pigs the findings were different, here, the epiphysial lines were patent and wider than under corresponding normal conditions. The cartilaginous matrix of the epiphysial line was loosened and swollen. Not only single cells, but not infrequently whole rows of cartilage cells, degenerated and disintegrated. But, on the other hand, proliferation of the resting and especially of the columnar cartilage cells had also taken place occasionally by way of mitoses. Instead of the ordinary number of ten columnar cartilage cells situated in one row, as many as twenty and more cells were counted, whereas the number of hypertrophic cartilage cells was apparently unchanged. In those instances in which there was predominance of proliferation of the cartilage over ossification, the trabeculae were not thickened. Here also a certain tendency of the bone marrow to undergo fibrosis was noticeable. In the joint the proliferation of the cartilage cells, in association with intensified processes of retrogression and solution, had led to the development of minute arthropathic lesions, which were more accentuated here than in the previous cases in which ossification predominated.

After two months of injections of the extract these two different modes of reaction were still present. In 3 of 5 animals the epiphysial line was distinctly narrowed (fig 1 *B*), and ossification of the cartilage was progressing. These 3 animals had gained only a little weight. The sclerosed cartilaginous ground substance had become more osseous, and the fibrils were thickened. The cartilage cell columns were irregular, in some areas replacement of cartilaginous tissue by bony material had occurred, which in places had led to the formation of osseous bridges between the epiphysis and the diaphysis (fig 2 *A*). Besides such bony bridges, wedgelike osseous plugs of varying length and thickness were seen. Not all these bony structures which had replaced the disintegrated cartilage cells persisted unchanged. Some of them were corroded and absorbed by constituents of the bone marrow advancing from the metaphysis, others underwent processes of solution by preserved neighboring cartilage cells, which continued to proliferate. The covering of the joint was thickened owing to the hyperplasia and increased ossification of the cartilage. In the 2 other animals, which had gained more weight, the epiphysial line was still of medium width, and proliferation of the cartilage cells was more accentuated than ossification of the cartilage. However, in contradistinction to the loosening and swelling of the matrix as observed after one month's injections of the extract, sclerosis of the cartilaginous ground substance and its fibrils was seen. In the joints there was thickening of the covering caused by intensive proliferation of the cartilage cells associated with processes of retrogression, solution and vascularization. In the bone marrow, ribs and vertebrae the changes were essentially the same as those found after one month.

After three or more months of treatment the two main types of reaction could still be recognized. Either the epiphysial line had become more and more ossified, and more osseous plugs appeared, connecting the trabeculae of the diaphysis with the epiphysis, while processes of solution of bone by cartilage cells and bone marrow could also be observed, or proliferation of cartilage cells was still proceeding, although at these later stages the individual cartilage cells were rather flat, the sclerosis of the cartilaginous matrix had increased, and ossification had set in in various places (fig 2 *B*). This twofold mode of reaction of the cartilage was reflected in the behavior of the bony tissues. Predominance of proliferation of the cartilage cells was associated with increased absorption and solution of bone

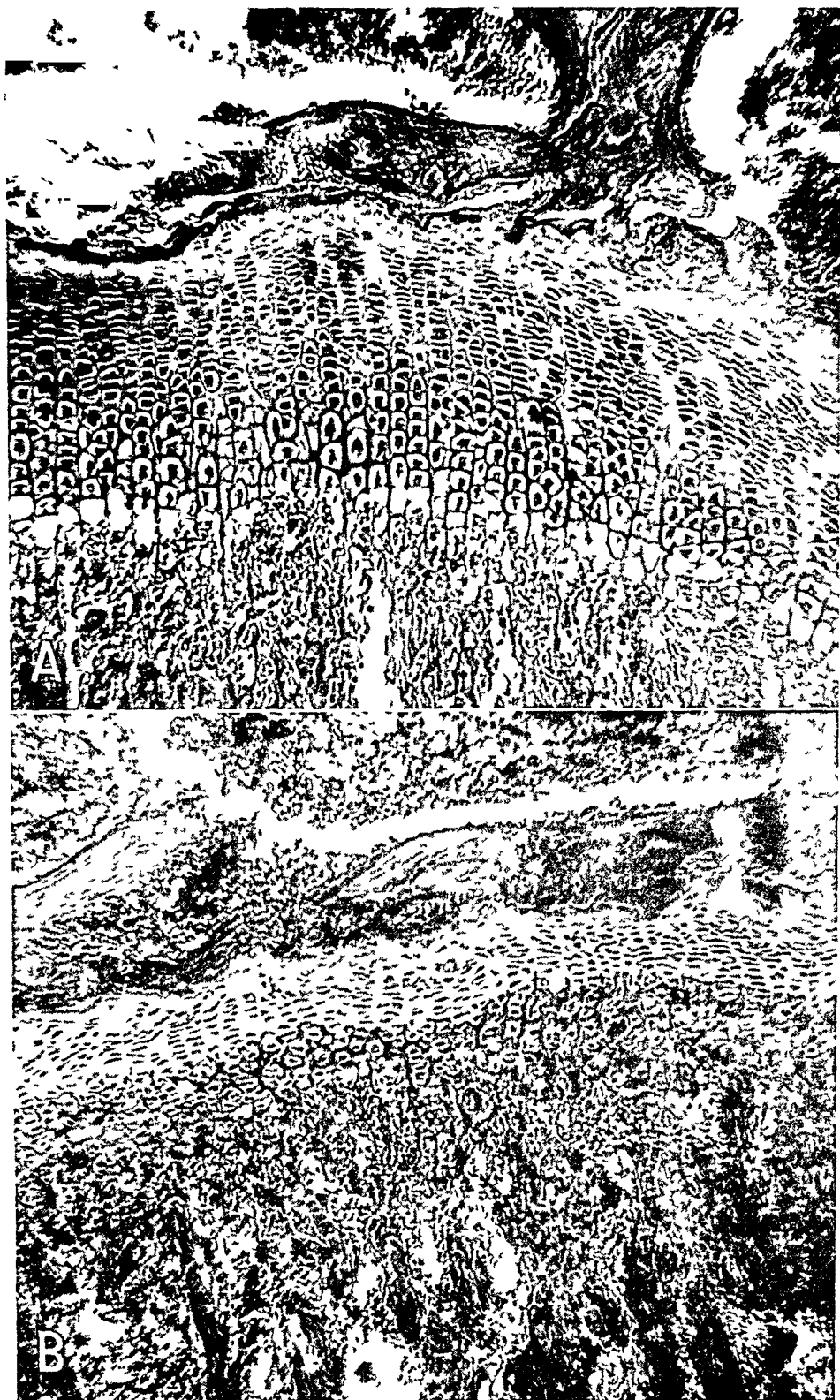


Fig 1—*A*, section through the upper part of a tibia of a normal female guinea pig weighing 275 Gm and of the same age as guinea pig 38. The epiphyseal line is of medium width, the cartilage rows are regularly arranged, there is no evidence of changes in the cartilaginous ground substance. Magnification, 150. *B*, section through the upper part of a tibia of a female guinea pig (38) which had shown an initial weight of 150 Gm and which received injections of 1 cc of anterior pituitary extract for two months. The final weight was 175 Gm. The epiphyseal line is narrowed, the columnar cartilage cells are diminished in number. A dense calcified layer of hypertrophic cartilage and thick bony trabeculae are seen. Magnification, 150.

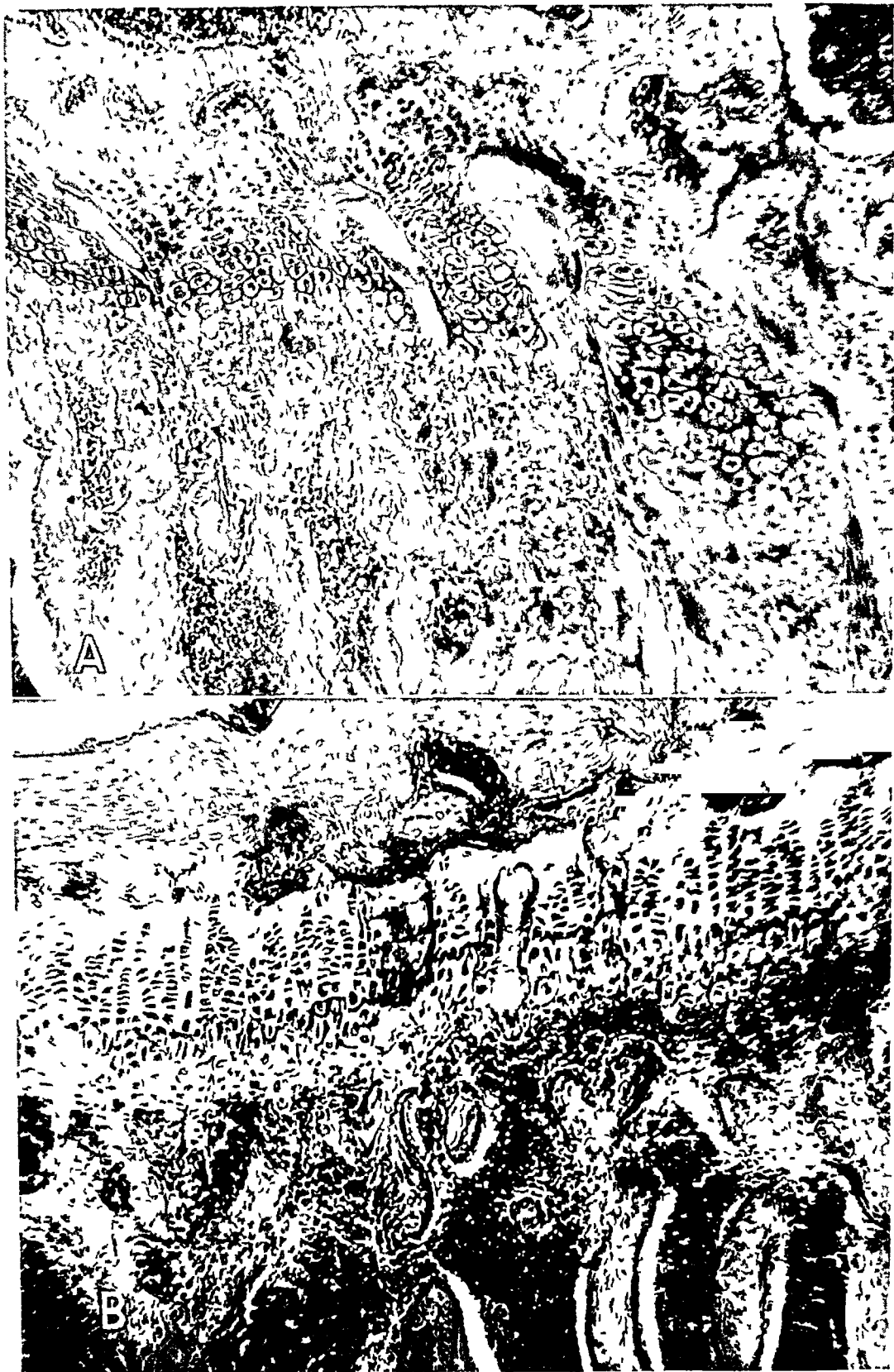


Fig 2—*A*, section through the upper part of a tibia of a female guinea pig (170/114) which had shown an initial weight of 175 Gm and which had been given injections of 1 cc of anterior pituitary extract daily for two months. The final weight was 275 Gm. The epiphyseal line is narrowed, four bony plugs have replaced destroyed cartilage cell rows, one plug is being invaded and dissolved by bone marrow. Magnification, 150. *B*, section through the upper part of the tibia of a female guinea pig (44) which weighed initially 145 Gm and which had been given injections of 1 cc of anterior pituitary extract daily for three months, subsequently the animal lived for four additional months. The final weight was 550 Gm. The epiphyseal line is of medium width, the cartilage cells are fairly numerous but irregularly arranged, three thick osseous plugs are seen traversing the epiphyseal disk and replacing cartilage cell rows, the matrix is sclerosed, the trabeculae are thickened. Magnification, 150.

This condition manifested itself in thinning and corrosion of the osseous border lamella of the joint as well as in the slender configuration of the bony trabeculae. Increased ossification of the epiphysial zone, on the other hand, was associated with deposition of more osseous substance in the trabeculae of the marrow, in the compacta of the shaft and in the covering of the joint. The thicker the compact bone of the shaft, the less numerous were the haversian canals and, correspondingly, the less marked were the resorptive processes which were observed.

In an animal which had been allowed to survive for four months after discontinuation of the injections there appeared three lines of transverse trabeculae parallel to the zone of ossification (fig 3). Between these trabeculae and the epiphysial line one could detect islands of nonossified mature euhyaline cartilage.

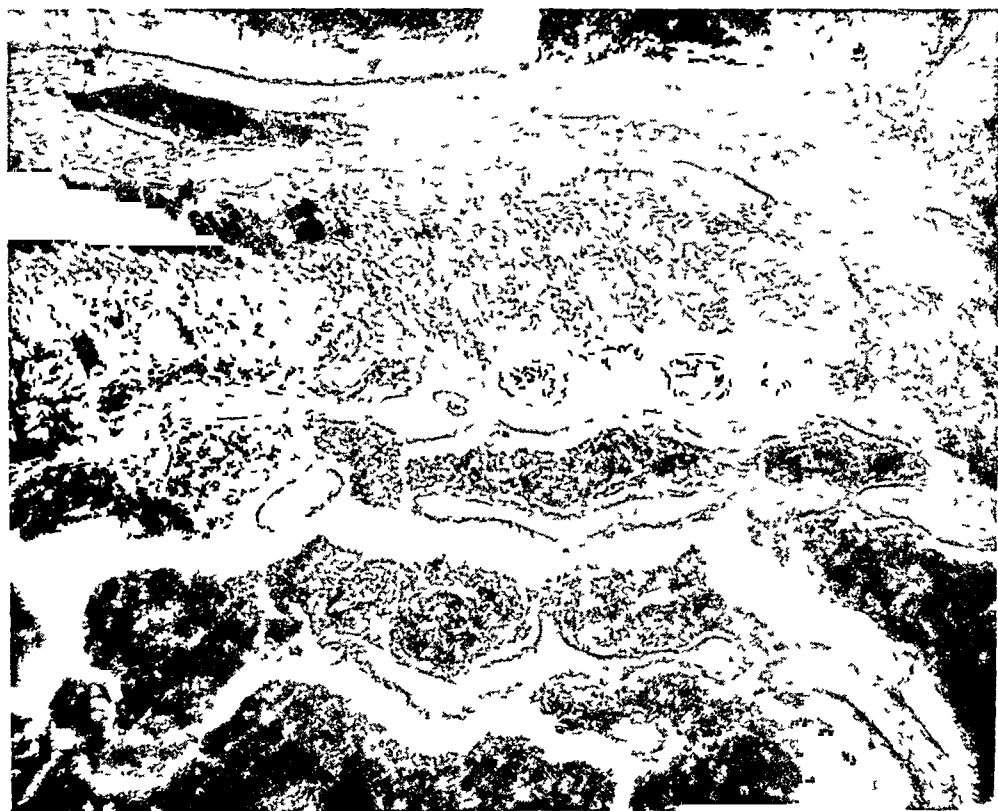


Fig 3—Section through the upper part of a tibia of a female guinea pig (43) with an initial weight of 140 Gm (same experiment as in fig 2B). The final weight was 535 Gm. The epiphysial line is fairly cellular and partly calcified. Note the presence of three lines of trabeculae with axes arranged in a transverse direction. Magnification, 120.

Table 3 gives concise descriptions of the changes in the weights of the guinea pigs and brief statements concerning the presence of so-called lines of arrested growth in their bones, furthermore, an attempt is made there to correlate the presence of lines of arrested growth<sup>5</sup> in the histologic section with periods of cessation of gain or of loss in weight.

<sup>5</sup> Harris, H. A. Bone Growth in Health and Disease, New York, Oxford University Press, 1933.

TABLE 3—*Summary of Observations on Relationship Between Cessation of Weight Increase and Lines of Arrested Growth in Bones*

Animal	Experiment	Histologic Observations as to the Presence of Lines of Arrested Growth	Weights
59	Extract for 6 mo	Some transverse trabeculae	Two definite periods of standstill 1st period, lasting 3 weeks, began 11 days after treatment was started, during which time gain in weight was good 2d period, lasting 1 week, during 3d and 4th months of experiment
59/119 (239A)	Extract for 4 mo	No line of arrested growth	Initial period of standstill lasting 12 days
64/119 (323A)	Extract for 4 mo	Few transverse trabeculae at some distance from the epiphysial line	Initial period of cessation of growth lasting 2½ weeks
64	Extract for 3 mo	No line of arrested growth	Initial standstill lasting 2 weeks, then slow growth up to the end of the 2d month, more rapid growth during 3d month
88/119 (110A)	Extract for 3 mo	No line of arrested growth	Initial period of standstill lasting 2 weeks 2d period of standstill lasting 1 week at end of 2d month
67	Extract for 2 mo	No line of arrested growth	Initial standstill lasting 1 week then slow gain during 1st month more rapid gain during 2d month
38	Extract for 2 mo	Transverse bony trabeculae directly adjoining the hypertrophic cartilage cell layer	Initial period of standstill lasting 3 weeks 2d period lasting 3 weeks during 2d month
74/119	Extract for 2 mo	Transverse trabeculae in metaphysis	No cessation of growth
170/119 (115A)	Extract for 2 mo	Transverse trabeculae at some distance from the epiphysial line	Initial period of standstill lasting 2 weeks 2d period lasting 1 week during 1st half of 2d month
171/114 (116A)	Extract for 2 mo	No line of arrested growth	Initial period of standstill lasting 2½ weeks
58	Extract for 1 mo	No line of arrested growth	Almost complete cessation of weight increase during time of experiment except for gain during 3 to 4 days
73	Extract for 1 mo	No line of arrested growth	Complete standstill for the initial period of 10 days, then slow growth
74	Extract for 1 mo	No line of arrested growth	Complete arrest for an initial period of 5 to 6 days, then good growth
143/114 (300A)	Extract for 1 mo	Few transverse trabeculae adjoining epiphysial line in the center	Initial period of standstill lasting 2 weeks
86/119 (137A)	Extract for 1 mo	No line of arrested growth	Initial period of standstill lasting 2 weeks
40	Extract for 4 mo, followed by 4 mo without injections	No line of arrested growth, marked ossification	Very slow gain in weight but no definite standstill during the 1st month, except for the 1st 4 days
43	Extract for 4 mo, followed by 4 mo without injections	Three definite transverse osseous lines	Three definite periods of standstill, 1st one at beginning of experiment lasting 4 days a 2d one lasting 2 to 3 weeks during 2d half of the 1st month and 1st half of the 2d month, and a 3d lasting 4 weeks during the 4th month, followed by a rapid gain
44	Extract for 3 mo, followed by 4 mo without injections	No line of arrested growth, very marked ossification	Initial period of standstill lasting 5 to 6 weeks 2d period lasting 2 weeks during the 4th month At this time weight almost identical with that of guinea pig 43, after discontinuation of injections rapid increase in weight

With the exception of 1 animal (74/119) all guinea pigs showed a period of cessation of weight increase immediately after the beginning of the injections. This period varied from four days to six weeks. In some cases, after this period of standstill, there occurred a continuous, if rather slow, gain in weight. In others (43 and 44) a slight gain in weight of about 15 Gm took place over a period of several days, followed by another period of standstill.

As to the parallelism between the cessation of gain in weight and the occurrence of lines of arrested growth in the microscopic sections, 3 cases are of particular interest. Although guinea pig 74/119 did not suffer any cessation of weight increase except for an early drop of 20 Gm within two days, still in the diaphysial marrow, instead of the ordinary longitudinal arrangement the axes of the trabeculae were definitely arranged in a transverse direction. On the other hand, guinea pig 44, in which cessation of gain in weight was most pronounced, did not reveal any evidence of such a line, on the contrary, it showed a very even distribution of osseous trabeculae, with their axes arranged in a longitudinal direction. Guinea pig 43 was the only animal in which three periods of cessation of gain in weight were accompanied by three definite transverse lines of osseous trabeculae in the marrow cavity.

Our findings are summarized in table 4.

TABLE 4—*Relationship Between Behavior of Weight and Lines of Arrested Growth in Treated Guinea Pigs*

Guinea Pigs	Number with Initial Loss of Weight	Number with Secondary Lack of Gain in Weight	Number with No Cessation of Gain in Weight	Number Showing Lines of Arrested Growth		Number Showing no Lines of Arrested Growth
				Pronounced	Trace	
18	16	6	2	1	6	11

From this summary it may be concluded that the occurrence of transverse trabeculae in the marrow cavities of guinea pigs under certain experimental conditions is not definitely correlated with the cessation of gain in weight in these animals.

#### COMMENT

The results obtained after prolonged administration of the acid extract of the anterior lobe of the bovine pituitary supplement and can be correlated with our findings in bone and cartilage after injections of this substance for shorter periods of time.<sup>6</sup>

Two types of reaction can be distinguished. The one is characterized by a predominating stimulation of the formation of cartilage, with com-

<sup>6</sup> With reference to a recent publication by J. Freud, L. H. Levie and D. B. Kroon (*J. Endocrinol.* **1**: 56, 1939) it may be mentioned that the extract used by us had been prepared according to the method of L. Loeb and R. B. Basset (*Proc. Soc. Exper. Biol. & Med.* **26**: 860, 1929), as stated in our previous reports (Silberberg, M. *Virchows Arch. f. path. Anat.* **289**: 201, 1933, footnote 1). Freud, Levie and Kroon stated, furthermore, that only limited histologic data are available. In several of our previous papers (footnotes 1 and 4b) we gave full histologic descriptions of our observations, which were illustrated by photomicrographs. Their paper is essentially confirmatory of our findings.

paratively little degeneration and ossification, and wide epiphysal zones, rich in cartilage cells. The second response consists of a proliferation of cartilage cells, associated with extensive degeneration in places and early replacement of the degenerated areas by bone. In the latter case the increased and premature ossification leads to narrowing and partial closure of the epiphysal disk.

The tendency of the extract to promote ossification finds expression also in thickening of the trabeculae, of the osseous parts of the covering of the joints and of the compact cortex of the long bones. This tendency, however, becomes apparent only at later periods. At earlier stages it is interfered with by increased resorption of osseous material, presumably under the influence of the hyperactivity of the thyroid gland which has been called forth by the anterior pituitary extract.

It was at first assumed by us that the disturbance of the balance between the new formation of cartilage and its ossification was due to differences in the degree of stimulation of these two processes. A higher degree of stimulation of cartilage growth would cause widening, while a stimulation leading to a relative and absolute increase in ossification would produce narrowing, of the epiphysal line. We could show, however, that proliferative processes in the cartilage were predominant in all instances at early stages of administration of the anterior pituitary extract.<sup>2</sup> Therefore, additional local or general factors must be decisive in determining the preponderance of ossification at later stages.

The occurrence of circumscribed osseous plugs in the epiphysal zone, especially in animals which had remained small and had not shown much gain in weight in the beginning of the experiment, gave some indication as to the nature of these factors. These bony enclosures had apparently replaced areas of degenerated cartilage and may therefore be interpreted as secondary processes and not as direct effects of the action of the extract. The degree of ossification in such instances apparently depends, therefore, on the occurrence of more or less extensive degenerations in the cartilage. This interpretation would be additional evidence for the view which we have previously expressed, namely, that ossification in general may be largely dependent on local factors which are active in addition to hormonal influences.<sup>7</sup>

An answer which would apply generally to the question as to why the cartilage is more prone to undergo retrogression in some instances than in others cannot as yet be given. We have been able to demonstrate<sup>4a</sup> that with advancing age the tendency of the cartilage to proliferate under the influence of the anterior pituitary hormone decreases, whereas its tendency to undergo retrogressive changes increases under this condition, but in the experiments which are under discussion the

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7 Silberberg, M., and Silberberg, R. Arch Path 28:340, 1939

differences in the mode of reaction are obviously due to other factors, because guinea pigs of the same weight and age and treated simultaneously with the same kind and dose of the extract responded to the latter in different ways. The tendency to form bone was, as a rule, greater in animals which had shown less gain in weight. A poor general condition may, perhaps, diminish the power of the cartilage to grow, at the same time intensify the degenerative processes initiated by the hormone, and thus increase bone formation.

In the epiphyseal lines of guinea pigs treated over longer periods of time the diffuse calcification of the cartilage which may be observed after injections for from one to three weeks was missing even in animals which had shown little gain in weight. However, the zones of endochondral ossification were narrow in those animals and osseous plugs indicated that degenerative processes had taken place. One might be inclined to consider these bony plugs traversing the epiphyseal zone in the direction from the metaphysis as permanent structures preventing in crossbar-like manner further growth of the bones in length. However, this interpretation is not necessarily correct. Some osseous bridges were found in animals which had gained weight steadily up to the time when they were killed, and there is no reason to assume that growth would not have continued if the guinea pigs had been allowed to survive. It is therefore probable that these bony plugs do not prevent further growth in length. Serial sections showed that they may form complete osseous bridges in some places, whereas they are being dissolved by ingrowing bone marrow or proliferating cartilage cells in other places. Resting cartilage cells and even well formed short columns of cartilage cells were seen proximally to the osseous plugs in these cases, the cartilaginous growth originated probably in preserved cartilage cells of the neighboring tissue. If the growth of the cartilage is still further stimulated, the remnants of the osseous plugs may be pushed downward in the direction toward the bone marrow, and may finally be incorporated into the mass of newly formed bony trabeculae of the metaphysis. But at the same time increasing sclerosis of the cartilaginous ground substance may lead to the production of new osseous plugs, and thus the competition between these two opposed processes persists. In the end an equilibrium between them may be restored, which guarantees the further growth of the animal in length, and this may counterbalance any shift of this equilibrium which might have occurred during the earlier stages of the experiment.

In those animals which had shown notable gain in weight throughout the duration of the experiments, the second type of reaction to the anterior pituitary extract, namely, a predominance of proliferation over ossification was seen even at later stages. Wide epiphyseal zones per-



sisted for comparatively long periods and the first symptoms of the formation of bony plugs did not set in earlier than four or more months after the beginning of the experiment

These differences in the histogenetic mechanism of the reactions of cartilage to the pituitary extract may explain the divergent results recorded by various investigators who studied the effect of anterior pituitary extract on body growth. In chickens Wulzen<sup>8</sup> observed arrest of growth for a period of three months after the beginning of the administration of the anterior pituitary substance. This was probably caused by a long-continued predominance of ossification over growth processes. Schafer<sup>9</sup> noted in rats, and Robertson<sup>10</sup> in mice a temporary inhibition of growth followed by normal or even increased growth. These findings agree with our own observations, in which a temporary shift of the balance in favor of ossification was followed by a restoration of the balance with higher rates of both proliferation and ossification. On the other hand, increased growth without initial inhibition as reported by Uhlenhuth<sup>11</sup> in salamanders, Howes<sup>12</sup> and Clements and Howes<sup>13</sup> in axolotl, Goetsch,<sup>14</sup> Evans and Long<sup>15</sup> in rats, Putnam and collaborators,<sup>16</sup> and Teel and Cushing<sup>17</sup> in dogs and Sousa Pereira<sup>18</sup> in rabbits might have been due to a predominance of proliferative processes over ossification for the greater part of the experiment. Finally, the negative findings of Smith<sup>19</sup> and of Larson and co-workers<sup>20</sup> do not exclude the possibility that histologic changes may actually have been present.

From the data of our experiments we cannot conclude that the stimulation of growth exerted by the anterior pituitary extract is maintained indefinitely. To us it seems more likely that the anterior pituitary extract acts in such a manner as to promote the proliferation of the cartilage and its ossification during parts of the physiologic growth

8 Wulzen, R. *Am J Physiol* **34** 127, 1914

9 Schafer, E. A. *Quart J Physiol* **5** 203, 1912

10 Robertson, T. B. *J Biol Chem* (a) **24** 385, 1916, (b) **24** 397, 1916

11 Uhlenhuth, E. *Anat Rec* **23** 43, 1922

12 Howes, N. H. *J Exper Biol* **15** 447, 1938

13 Clements, D. I., and Howes, N. H. *J Exper Biol* **15** 541, 1938

14 Goetsch, E. *Bull Johns Hopkins Hosp* **27** 29, 1916

15 Evans, H. M., and Long, J. A. (a) *Anat Rec* **21** 62, 1921, (b) *Proc Nat Acad Sc* **8** 38, 1923

16 Putnam, T. I., Teel, H. M., and Benedict, E. B. *Am J Physiol* (a) **84** 157, 1928, (b) **85** 40, 1928

17 Teel, H. M., and Cushing, H. *Endocrinology* **14** 157, 1930

18 Sousa Pereira, L. *Lyon chir* **34** 673, 1937

19 Smith, C. S. *Am J Physiol* **65** 277, 1923

20 Larson, E., Bergeim, O., Barber, D. I., and Fisher, N. I. *Endocrinology* **13** 63, 1929

period of the animal and to produce acceleration in the maturation of the skeleton in those animals in which ossification predominates. On the other hand, maturation of the skeleton is delayed if the rate of ossification lags behind that of the intensified new formation of cartilage. The fact that injections of the extract when given over a long period did not produce increased growth beyond the normal adult size, leading to gigantism, may depend on various factors. With increasing time of administration the extract may lose its ability to stimulate tissues, as shown in the case of the ovaries and thyroid by Loeb,<sup>21</sup> this loss of stimulating effect may depend on a gradual change in the reactivity of the epiphyseal cartilage under the influence of long-continued administration of the extract.

#### SUMMARY

Prolonged administration of an acid extract of the anterior lobe of the bovine pituitary exerts a stimulating action on (1) the proliferation and (2) the ossification of cartilage. These effects are similar to those which were noted after administration of this extract for shorter periods of time. The balance between proliferation and ossification may be disturbed in favor of one or the other of these two processes, and this effect may last for some time. If the rate of ossification of cartilage predominates over the rate of new formation of cartilage cells, an acceleration in the maturation of the skeleton may take place, but if the rate of proliferation of cartilage predominates over that of ossification, maturation of the skeleton may be delayed. However, the balance between these two processes can be restored at later stages. The reestablishment of this balance is responsible for the adjustment of skeletal growth. The course of ossification is influenced not only directly by the administration of the anterior pituitary extract but also indirectly by the occurrence and the severity of local retrogressive processes taking place subsequent to the administration of the extract. The histologic differences in cartilage and bone caused by the predominance of proliferation or of ossification of cartilage and the subsequent restoration of a balance between the latter would account for the divergent observations on body growth as reported by different investigators. So-called lines of arrested growth can be observed in the guinea pig, but they do not necessarily coincide with the periods of cessation of weight increase which may have occurred.

Mr S J Hayward made the photomicrographs

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21 (a) Loeb, L. *Science* **80** 252, 1934. (b) Max, P., Schmeckebier, M. M., and Loeb, L. *Endocrinology* **19** 329, 1935.

# MALIGNANT ADENOMAS OF THE CHROMOPHOBE CELLS OF THE PITUITARY BODY<sup>3</sup>

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There has been much discussion in recent years among clinicians and pathologists in regard to the classification of the more extensive and invasive tumors arising from the chromophobe cells of the pituitary body. Small adenomas taking origin in these cells are not infrequently found in routine postmortem examinations<sup>1</sup>, these usually cannot be correlated with any clinical signs when the patient's history is reviewed. If the tumors of the chromophobe cells of the pituitary body are arranged in a series according to size, they pass from such incidental lesions through tumors causing varying degrees of visual disturbances up to huge neoplasms which destroy the optic chiasm, the normal pituitary tissue and adjoining portions of the skull and cerebral tissue. But does this series represent a single type of tumor? Certainly there is no justification for making subdivisions in a series if size is the only variable component. Or are there among these tumors neoplasms which are sufficiently distinct to warrant separate classification? If there are malignant chromophobe adenomas of the pituitary body, it would be desirable to establish criteria for their identification in the roentgenogram and biopsy specimen, since the accumulation of such data might lead to an appreciation of the special problems in treatment to which these tumors give rise.

The malignant chromophobe cell adenomas have been the subject of relatively few papers, considering the divergence of opinion which has been expressed from time to time by those interested in the subject. As a part of a paper dealing with clinical and pathologic aspects of hypophysial adenomas, Dott, Percival Bailey and Cushing<sup>2</sup> mentioned the malignant adenoma as one of the types of tumors arising from

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1 Parsons, R, in Medical Papers Dedicated to Henry Asbury Christian, Baltimore, Waverly Press, Inc, 1936, p 336

2 Dott, N, Bailey, P, and Cushing, H Brit J Surg **13** 314, 1925

chromophobe cells and discussed briefly 3 examples, 2 of which were studied in biopsy material only, in the case of the third necropsy was performed. The short statement by Fink<sup>3</sup> contains more valuable information on this subject than many of the longer discussions. Some of the other papers dealing with this question are those of Kux, Redlich and Maxted<sup>4</sup>. Salus<sup>5</sup> described a woman who displayed many of the characteristics of Cushing's syndrome for several years. Eventually she lost ground and died without exacerbation of the symptoms of Cushing's syndrome. At autopsy Salus found a large tumor invading the nasopharynx. The tumor cells did not contain basophilic granules. Was this a basophile adenoma with malignant degeneration? Did the patient have first a basophile adenoma and later an independent malignant chromophobe adenoma which destroyed the first? It is impossible to say. There is little similarity between Salus' interesting case and those dealt with in this paper. The discussion of the possibility of chromophile cell carcinoma falls outside the scope of this paper.

In a study recently published from this clinic<sup>6</sup> the instances of chromophobe adenoma of the pituitary body encountered between Jan 1, 1928, and Jan 1, 1936, have been discussed. These amounted in all to 88, 81 of which were verified by biopsy or postmortem examination, and 7 of which were studied only clinically and in roentgenograms. The 81 verified tumors were divided histologically into diffuse and sinusoidal types, a classification suggested by Dott, Percival Bailey and Cushing,<sup>2</sup> which was elaborated, illustrated and correlated with clinical data by us. After this material had been studied, there remained 3 tumors which appeared different from the others. We felt that they should be regarded as a special group to be set apart from the ordinary chromophobe adenomas because of the unusual clinical histories of the patients and the invasive properties and distinctive histologic appearance of the tumors. It may seem to lay undue stress on so small a group to devote a special publication to it. Yet a detailed consideration of the 3 patients and a study of the tumors in the light of general oncologic principles may contribute toward the understanding of a lesion which is one of the most difficult to identify and to treat of any affecting the pituitary body.

#### CASE 1

*Two year history of visual disturbances, impotency, seizures with olfactory prodromes. Extensive destruction of sella turcica shown in roentgenograms. Trans-*

3 Fink, E. B. *Tr. Chicago Path. Soc.* **12** 359, 1927.

4 (a) Kux, E. *Beitr. z. path. Anat. u. z. allg. Path.* **87** 59, 1931. (b) Redlich, F. *Wien. Arch. f. inn. Med.* **30** 111, 1937. (c) Maxted, G. *Proc. Roy. Soc. Med. (Sect. Ophth.)* **12** 42, 1919.

5 Salus, F. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **148** 574, 1933.

6 Schnitker, M., Cutler, E., Bailey, O., and Vaughan, W. *Am. J. Roentgenol.* **40** 645, 1938.

*frontal craniotomy followed by intensive roentgen therapy Patient alive and at work six and one-half years after operation Tumor histologically malignant chromophobe adenoma*

Mr J T first came to the clinic Feb 17, 1933, at the age of 40, because of gradual loss of vision This had made its first appearance in 1931 In the beginning the difficulty was confined to impairment of vision in the temporal field of the right eye, but there was a steady increase in extent, which was only temporarily improved by successive changes of eyeglasses In November 1932 the patient found that when he drove his automobile an approaching vehicle would disappear from sight as it came opposite his car The patient then began carrying out crude visual tests, which showed absence of vision in four fifths of the right visual field and blurred vision in the remainder of that field He also found that there was no vision in one fifth of the left temporal field of vision Soon after this he began to experience transient attacks of diplopia lasting fifteen to thirty seconds whenever he turned his head suddenly The visual disturbances progressed in severity to such an extent that the right eye was almost blind and vision in the left eye was seriously impaired when he came to the hospital

While at work in August 1932 he had an attack of weakness and dizziness, at the same time he thought he smelled coal gas He went home at once and fainted shortly after arriving there Complete unconsciousness lasted for an unknown interval The patient was not incontinent at the time On the next day he again stated that he smelled coal gas, but those about him could detect no such odor The olfactory sensation was followed by a sense of faintness without loss of consciousness There were four more of these attacks up to December 1932

In that month, mild headaches in the frontal region made their appearance These occurred every day and were associated in the patient's mind with eyestrain At intervals there were also more severe occipital headaches The patient had no attacks of vomiting, there was no epistaxis or rhinorrhea He had maintained a weight of 202 pounds (91.5 Kg) His height was 176 cm He had 4 normal children Libido was absent during the two years previous to his admission to the hospital

*Examination*—The patient was alert, well developed and stout, the fat over the abdomen and hips being somewhat more noticeable than elsewhere The right pupil was larger than the left and reacted sluggishly to light and accommodation The left showed normal reactions in both tests There were no abnormalities of movement in the extraocular muscles The genitalia were well developed, and the body hair followed the distribution normal in men The tendon reflexes were physiologic with the exception of the right ankle jerk, which was hyperactive All test odors were recognized Studies of the visual fields showed almost complete left temporal hemianopia There was a small area on the nasal side of the right visual field in which vision was preserved, but even the largest test disk could not be perceived by other parts of the right visual field The corrected acuity of vision was 20/40 in the left eye, 5/200 in the nasal field of the right eye, and 0 elsewhere Examination of the right optic fundus disclosed a pale optic disk with well defined margins There was a deep physiologic cup and a visible lamina cribrosa The left optic fundus was similar except that the optic disk was not so pale The blood pressure was 135 mm of mercury systolic and 85 mm diastolic The basal metabolic rate was minus 17 per cent The blood and urine were essentially normal

*Roentgen Observations*—The cranial vault was rather thick and dense The sella turcica was almost completely destroyed, but the right anterior process

remained. The left anterior clinoid process was not visualized. There was no evidence of a soft tissue mass in the nasopharynx (fig 1). Roentgenograms of the right hand and wrist showed large bones but no acromegalic changes. The destructive lesion in the sella turcica was regarded as suggestive of a malignant tumor rather than of the usual type of chromophobe adenoma.

*Operation*—Feb 21, 1933, a right frontal bone flap was turned down. The frontal bone was thickened but did not have the appearance of the skull in



Fig 1 (case 1)—Roentgenogram showing almost complete erosion of the sella turcica. The amount of bone destruction is greater than that usually seen with chromophobe adenoma of the pituitary.

acromegaly. When the pituitary fossa was brought into view, it was seen to be filled with a meatlike tumor, which flattened the right optic nerve, pushing it upward and outward. Some large fragments were removed for histologic study, and more tumor tissue was evacuated with suction.

*Subsequent Course*—Convalescence was rapid and without untoward incident. The visual fields March 8 showed a slight contraction of the defect in the left eye. The patient could distinguish an object the size of the examiner's hand in

the area which was blind before operation. He was discharged March 16. Roentgen therapy was started March 11, between that date and March 15 four treatments, each of 55 per cent of a Holzkecht erythema dose, were given alternately to the right and left temporal portals. The visual fields April 26 showed so much improvement in the left eye that only a small defect remained in the upper temporal quadrant. With the right eye the patient could see the examiner's head on the nasal side of the field but could not recognize the test disks. He felt stronger and the improvement in vision (registered objectively in the visual field charts) enabled him to read better than before operation. Four more roentgen treatments of the same character were given.

June 26, a little more than three months after the patient left the hospital, his visual fields and general condition remained essentially unchanged. He was given another series of four roentgen treatments, the dose being the same as before. After that the patient remained symptom-free except for the defect in vision in the right eye. Since he had no further symptoms, he could not be persuaded to return regularly for observation. In December 1934 he found blood on the pillow when he awoke, which led him to think that he might have had a convulsion during the night. He then returned to find out the cause. The visual fields had remained almost exactly as they were in April 1933. Further roentgen therapy was recommended but refused. One day at the end of March 1935 he suddenly lost consciousness and was told that he had had a convulsion. There was another less severe convulsion a few days later. Because of these, he reentered the hospital on April 6, 1936. Physical and neurologic examinations showed conditions unchanged from those at the time of the previous admission. Roentgenograms of the skull showed that the extent of destruction of the sella turcica had altered little in the three years since the patient first came to the hospital. The left visual field was unchanged from that seen in April 1933. There was perception of light only in the right eye. Roentgen therapy to the amount of 1,170 roentgens was given to each temporal area. A fifth series of roentgen treatments was given in June because of another convulsion and several "fainting attacks." The total dose to each temporal area was 900 roentgens. Repeated visits to June 1937 showed no further change in visual fields and no untoward symptoms. Another series of roentgen treatments was given at that time, the total dose to each temporal region being 1,050 roentgens. Roentgenograms of the skull in June 1938 showed some regeneration of the anterior clinoid processes on the right and slight but definite evidence of healing in all the region of the destroyed bone. At the last examination (February 1939) further improvement in the fields of vision was noted, and no further seizures or headaches had appeared. The patient's weight was 220 pounds (100 Kg), he remained impotent. He was able to carry on his work as traveling salesman without trouble and had no difficulty with the automobile driving required by it. Roentgenograms of the skull taken at that time showed the pituitary fossa to be about the same size and shape as noted at the first examination, six years previously. The posterior clinoid processes were partly eroded, the right anterior clinoid process was thinned and displaced laterally, while the left was eroded. The left spheno-orbital fissure was larger than the right. The patient received no more roentgen treatment after June 1937.

*Biopsy*—The material consisted of 3.5 Gm of grayish pink tissue in large fragments. It was soft and pulpy. This material was fixed in Zenker's fluid with 5 per cent acetic acid, embedded in paraffin and stained by the following methods: hematoxylin-eosin, eosin-methylene blue, Mallory's phosphotungstic acid-hematein and Foot's modification of the Bielschowsky-Maresch method for reticulum with Van Gieson's stain as a counterstain.

Histologically, the cells of the tumor were massed in broad irregular sheets separated from one another by delicate strands of collagenous stroma. There were many capillaries scattered among the clusters of tumor cells. Most of the strands of stroma were associated with the capillaries, and the collagen seemed rather refractile. The round or oval nuclei of the tumor cells were rich in chromatin. The cytoplasm stained pale and contained very fine granules of a neutral staining reaction. No chromophile cells were encountered. There was moderate variation in cell size and shape from place to place. Mitoses were scattered throughout the tumor in moderate numbers (fig 2). In one fragment

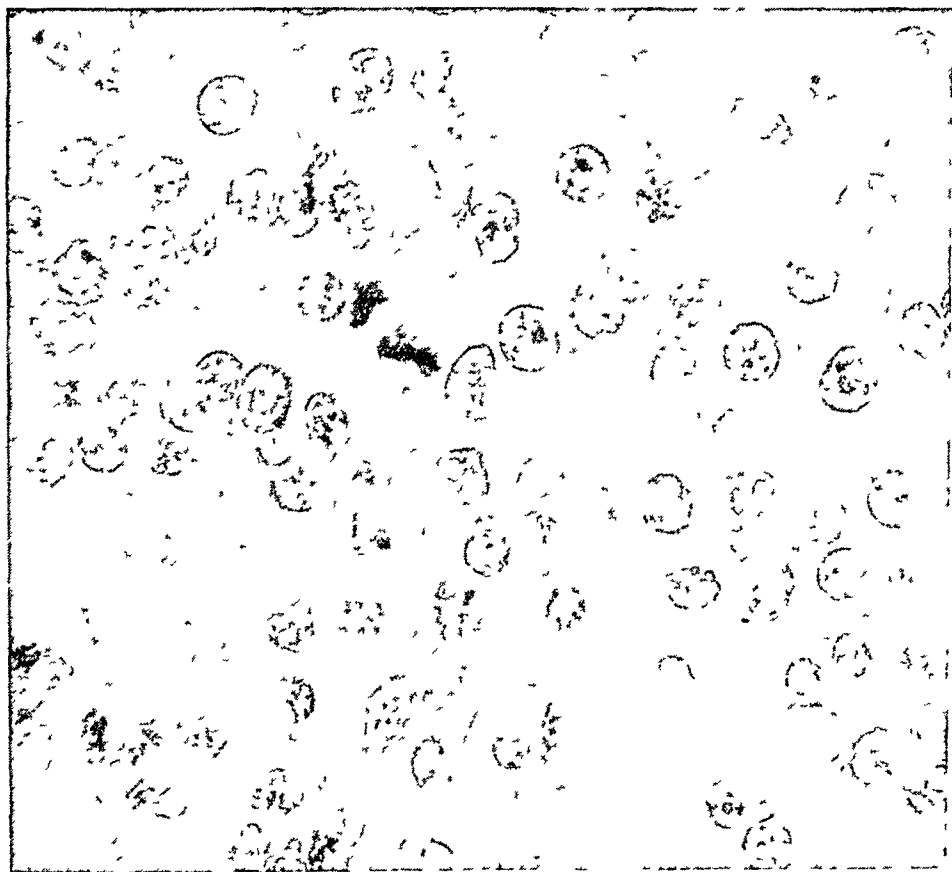


Fig 2 (case 1)—Photomicrograph of a field in the material removed at operation. The chromophobe cells are closely packed, at the center is a mitosis. Hematoxylin and eosin,  $\times 900$ .

of the tumor mitoses were especially numerous, two being seen in a single high power field on several occasions. The edge of the tumor was not included in the biopsy material.

#### CASE 2

*Nine months' history of headache, failing vision, convulsions and aphasia. Anosmia, large defects in fields of vision, bulbar paresis. Erosion of sella turcica shown in roentgenograms. Exploration of left temporal lobe with fatality. Autopsy: malignant chromophobe adenoma with extension into left temporal lobe and sphenoid sinus.*



Mr R P was admitted to the hospital Jan 2, 1934, because of headache and difficulty in vision. His difficulties had begun in May 1933, with a steady, burning headache, situated, as he said, "behind the eye." The pain began in the morning and lasted all day, salicylates gave no relief. In the following June he first noticed slight difficulty in vision, but this did not become a major symptom. One month later the patient had an attack of stertorous breathing and could not be made to respond for an hour. The attack was not accompanied by a convulsion, and there were no speech disturbances or other residua after consciousness returned. In October a similar attack was followed by difficulty in speech so severe that he could not be easily understood. He was not able to name objects quickly, though his celebration seemed otherwise normal. A third attack occurred in November. During this seizure he did not lose consciousness but was unable to speak for about three hours. Speech was then produced in the thick, poorly articulate manner which it had assumed after the second seizure. In December all the patient's teeth were removed. On the next night there was a generalized convulsion, consisting of jerking movements of both arms and legs. The patient was semicomatose during the convulsion and felt drowsy for the next two days. Several similar convulsions occurred during the following week. Nocturia then appeared for the first time. At this period, also, the patient would frequently drop his knife or fork and would be unable to grasp the utensil firmly in either hand when it was returned to him. Walking became troublesome at this period because he could not lift his feet and was compelled to drag them after him. Complete paralysis was not present at any time. Vomiting occurred only once, and then following medication. At the time the weakness of both arms and legs developed, some difficulty in swallowing liquid appeared, and on one occasion regurgitation through the nose followed a fit of coughing. There was at no time evidence of epistaxis or rhinorrhea. The patient had gained 30 pounds (13.5 Kg) in the previous ten years. He was married fourteen years before, there were 4 normal children. No recent change in libido had been noted.

*Examination*—The patient was well developed, somewhat obese, drowsy and in no acute distress. The right pupil was slightly larger than the left, both reacted normally to light and to accommodation. There was weakness in conjugate movements of the eyes to the left. A slow nystagmus was noted when the patient looked either to the right or to the left. There was choking of both optic disks of 1 to 2 diopters, with considerable blurring and hyperemia at the periphery. The visual acuity of the right eye was 20/70, that of the left, 20/100. Charting of the visual fields disclosed nearly complete temporal hemianopia on the right, on the left there was only a small area of vision remaining at the center, since both the temporal and nasal fields were almost completely obliterated. There was normal response to test odors on the right, but complete anosmia was found on the left. There was a suggestion of weakness of the right lower facial musculature. The lower jaw could be only partially opened. The soft palate deviated to the left. Speech was thick and blurred, the patient hesitated for some time before saying the name of any common object. All tendon reflexes were hyperactive but equal on opposite sides. Hoffmann's sign was present on the right but the Babinski sign was not. The hair on the body followed the male type of distribution, the genitalia were normal in development. The blood pressure was 130 mm of mercury systolic and 80 mm diastolic. The blood and urine were normal. The spinal fluid was under slightly increased initial pressure. It was yellow and gave a strongly positive Pandy reaction.

*Roentgen Observations*—Right stereoscopic and anteroposterior views of the skull showed a vault of average thickness. The pituitary fossa measured 18 mm

in length and 15 mm in depth. The left anterior clinoid process was destroyed, and both posterior clinoid processes were rarefied. The left half of the sella turcica was completely eroded (fig 3). The pineal body occupied a position to the right of its normal situation.

Ventriculograms showed both lateral ventricles displaced to the right of the midline. The left temporal horn was displaced upward and backward. The third ventricle was not definitely outlined, but the air passed freely between the lateral

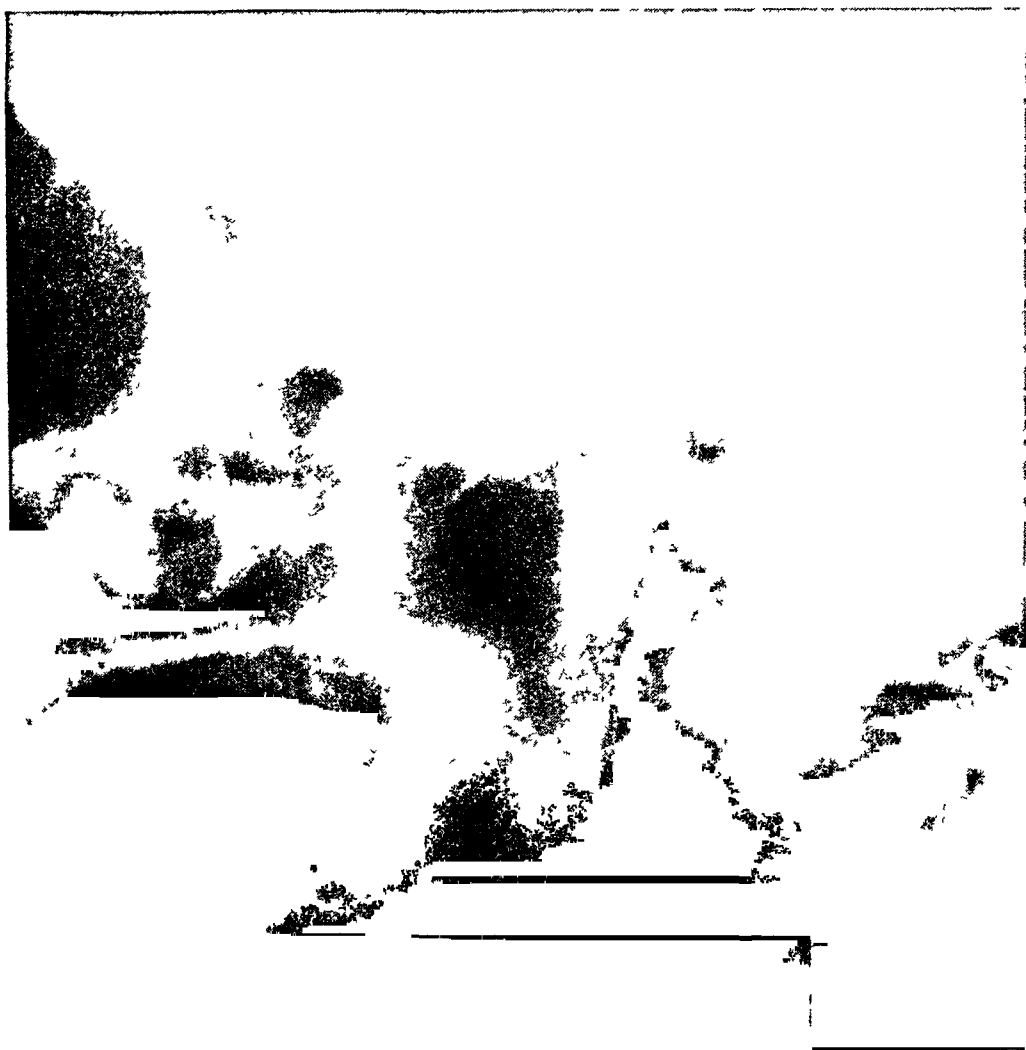


Fig 3 (case 2)—Roentgenogram showing great enlargement of the sella turcica. The left anterior clinoid process has been destroyed, and the posterior clinoid processes are rarefied.

ventricles. The right ventricle was slightly dilated but was otherwise normal in appearance. This was regarded as indicative of a tumor of the first temporal lobe, probably an extension from a pituitary adenoma.

*Operation*—The procedure began with a ventriculographic examination, the results of which have been given. An osteoplastic bone flap was then turned down in the left temporal region, and the area of dura thus exposed was enlarged

by removal of bone as far as the floor of the middle fossa. The dura was under great tension. The convolutions which had been brought into view were flattened and the delimiting sulci almost obliterated. An incision was then made in the temporal cortex at the posterior margin of the field. After about 2 cm of relatively normal cortical tissue had been passed through, a grayish red tumor of great vascularity was met, it was bleeding actively. To the operator's



Fig. 4 (case 2) —Photograph of the base of the brain showing the enormous tumor occupying the pituitary region, extending backward over the pons and into the left temporal lobe. The operative defect in the left temporal cortex exposes the deeper portions of the tumor in that location.

examining finger, the tumor seemed well demarcated, lobulated and soft in consistency. Four grams of neoplasm were enucleated. It was then apparent that there was an attachment of the tumor to the base of the brain in the midline near the middle fossa. Further removal of tumor was impossible. The exposed surface of the neoplasm was accordingly seared with the electric cautery, the wound

washed several times with saline solution and the dura reapproximated, leaving a large decompression in the middle fossa. Before the operation the patient was comatose, there was little change at its conclusion.

*Postoperative Course*—The patient's condition remained precarious after the operation. It became apparent that the speech center had been affected by the operative procedure, since he could understand and execute simple commands but could not speak. The condition of the patient did not show any definite improvement. On the eighth postoperative day the throat from time to time filled with mucus, but little other change occurred. Then quite suddenly that evening respirations ceased.

*Necropsy*—Examination was restricted to the head. When the surface of the brain was exposed by reflection of the dura, the convolutions appeared more flattened in the left hemisphere than in the right. A soft tumor was palpated in the left temporal lobe, this was located beneath the area of maximum convolutional flattening. When the basilar surface of the brain was inspected, attention was at once directed to a reddish gray, extremely soft tumor extending into the left temporal lobe from the region normally occupied by the pituitary body (fig 4). The mass in the region of the sella turcica measured 5.5 by 5.5 cm, the medulla was partially encircled by a backward extension, 4 cm in transverse extent and 2 cm in anteroposterior diameter. There were many irregular excrescences jutting out from the main tumor. It was an unusually large one of these which produced a tumor bridge to the left temporal lobe, where it formed the mass surgically attacked. The optic chiasma was pushed far to the right of the midline and was partially included in the mass of tumor. The left trigeminal nerve passed directly through the neoplasm and was separated from it with difficulty. The left semilunar ganglion was enlarged to 6 mm in diameter and was partly surrounded by tumor. Considerable dissection was required to find the rest of the cranial nerves on the left, since all of the first nine either passed through the tumor or had their course altered by it. The cranial nerves on the right side were normal.

The base of the skull was then studied (fig 5). The cribriform plate of the ethmoid bone was very thin, but there was no extension of tumor to the ethmoid sinus. The frontal sinuses were normal. The sella turcica measured 3 cm transversely and 2 cm anteroposteriorly. The right wall was thin and convex in outline. Elevation of the dura in this region uncovered a subdural extension of the tumor, 1 cm in thickness and 2 cm in extent, both anteroposteriorly and laterally. There was an irregular anterior extension over the great wing of the sphenoid bone, which resulted in rounding off the left anterior clinoid process and in pushing the right anterior clinoid process farther to the right than normal. Both posterior clinoid processes were also involved. The interior of the sphenoid sinus contained soft, friable, grayish red tumor. There was a small communication between the sinus and the sella turcica to the right of the midline. The petrous portion of the left temporal bone was involved by tumor for 3 cm adjacent to the sella turcica, but the lateral 4 cm was free from tumor. The neoplasm extended backward to the occipital bone, which it overlay rather than invaded. The posteriormost tip of the tumor was just anterior to the foramen magnum. The venous sinuses of the dura were free from neoplasm and thrombi. The left internal carotid artery was embedded in the tumor but was not compressed or thrombosed. Careful investigation of the nasal cavity showed it to be free from neoplasm.

After fixation in solution of formaldehyde U. S. P. diluted 1:10 (4 per cent formaldehyde), a coronal section was made, passing approximately through the

center of the brain so as to divide the left semilunar ganglion and the tumor into equal parts and to pass through the third ventricle and basal ganglions (fig 6) The left lateral ventricle was somewhat compressed, and the left basal ganglions were pushed upward as a whole, the left putamen and globus pallidus were destroyed by the tumor. The left thalamus and internal capsule were slightly distorted by the general upward dislocation of structures by the neoplasm and were infiltrated by the tumor for a short distance inferiorly. The right basal ganglions were normal. A small amount of changed blood covered the ependymal surfaces. The diameter of the third ventricle did not exceed 1 mm at any point. It was pushed to the right of the midline and curved along the surface of the tumor, but its ependymal surface was free from neoplasm. The only portion of the cerebral cortex affected by the tumor was the left temporal lobe. The operative

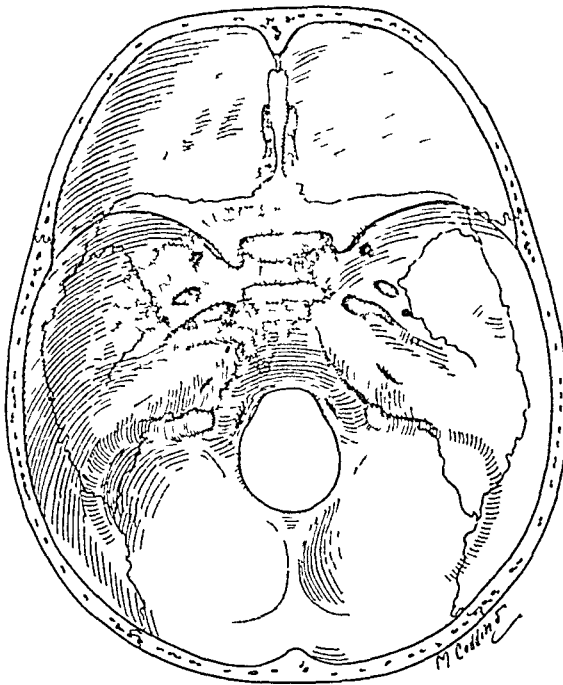


Fig 5 (case 2)—Diagram to show the extent of bone erosion (stippled). By comparison with figure 4, it may be seen that some of the tumor lay on the surface of intact bone but that the area eroded was large.

incision in this region extended 3 cm into the cortical tissue. A considerable portion of the subcortical neoplastic mass remained. It was evident that the tumor in the temporal lobe, that in the basal ganglions and that in the sella turcica were directly continuous (fig 6). The mass in the region of the pituitary body measured in cross section 5 by 4 cm. While most of the tumor surface was dark, irregular and mottled, there were a few white areas, especially along the lower margin, which were firmer than the surrounding tissue. The remainder of the neoplasm was soft even after fixation and showed very extensive degeneration.

*Microscopic Examination*—The tumor was examined by the histologic techniques listed as used in case 1. On the whole, the type cell of the neoplasm resembled the chromophobe cell of the normal pars anterior. The nuclei were round or slightly ovoid, the nuclear membranes were distinct, and there was a moderate

amount of chromatin. Occasional mitotic figures were found throughout the tumor. The cytoplasm of the type cell took a very pale stain, and on inspection with the highest power microscopic objective it was found to contain many fine granules, which were neutral in staining reaction. The cell walls were not distinct. There was considerable variation in cell size, with a general tendency for the cells to be larger than normal chromophobe cells. No chromophile cells were found in the tumor itself. A very few tumor cells contained small cytoplasmic vacuoles. On the whole the tumor was highly cellular, the cells being arranged in very large groups. The stroma in most areas was composed of small blood vessels and very delicate strands of connective tissue. There was no constant relationship between cells and stroma, and nothing resembling the sinusoids of the normal pars anterior was seen. In some areas, however, there was coarser



Fig 6 (case 2) —Coronal section of the brain exposing the tumor in its largest cross section. Note the destruction of most of the left basal ganglions and the distortion of the remainder. This photograph shows that the tumor spread entirely by direct extension.

and more richly collagenous connective tissue. While there were in some places bits of coarse connective tissue from the surrounding dura included within the tumor, the areas with the altered stroma were distinct and the connective tissue newly formed. The new connective tissue appeared to be derived in part, however, from the dura, for when some of the strands were traced out, they could be seen to spread through the tumor from a bundle of dural connective tissue, like the ribs of a fan. In other places, the new stroma originated in the adventitial connective tissue of the larger arteries, an especially good example being seen in connection with the internal carotid artery. The tumor compressed the edge of this vessel and derived some of its stroma from the adventitia, but it did

not infiltrate the inner portions of this layer or affect appreciably the media or intima. The tumor did not grow into the lumens of blood vessels.

The region of the gasserian ganglion and the adjacent portions of the trigeminal nerve were studied. The nerve cells were preserved, but the general architecture of the ganglion was considerably disrupted by the tumor. Masses of neoplastic cells extended into the ganglion and divided groups of nerve cells from one another with little necrosis of nerve tissue. The bundles of nerve fibers in the motor portion were widely separated from one another by tumor, but the individual fibers appeared intact (fig 7).

Throughout the tumor, but especially in the temporal portions, there were extensive necrosis and hemorrhage. The edge of the tumor where it bordered on basal ganglions and cerebral cortex was sharp. There was little glial reaction in the adjacent cerebral tissue.

An atrophic fragment of the normal pars anterior was included in the material. The sinusoids were compressed and narrow. Well preserved cells of all the normal types were found. However, many of the eosinophils contained small cytoplasmic vacuoles. At the junction of the neoplasm and the fragment of pituitary the tumor cells infiltrated the bit of pars anterior for a short distance.

### CASE 3

*Ten months' history of nasal obstruction, visual disturbances and headache for six months, diminished sense of smell and amenorrhea. Intranasal growth removed elsewhere, which proved to be extension from pituitary tumor. Complete bitemporal hemianopia, destructive lesion of sella turcica shown in roentgenograms. Transfrontal craniotomy with operative fatality. Autopsy: malignant chromophobe adenoma with invasion of base of skull, sphenoid sinus and right nasal cavity.*

Miss Z. H. was a Turkish school teacher, 31 years of age, who came to this hospital for treatment May 16, 1933, after a diagnosis of chromophobe tumor of the pituitary body had been made by her physician in Angora, Turkey.

The first hint of the series of misfortunes which was to befall her was a slight difficulty in breathing which developed in July 1932. Investigation showed that this was due to nasal obstruction. As time went on, this obstruction increased. In March 1933 a surgeon in Angora removed a polyp in an endeavor to improve the condition. On microscopic examination the polyp proved to be a pituitary adenoma of unusual histologic character. Between that time and the time of the patient's arrival at Boston the nasal obstruction increased still further. It was this difficulty in breathing which caused her the most distress throughout her illness.

There was, however, a variety of other symptoms. Beginning in November 1932, she noted a rather severe blurring of vision and a decrease in visual acuity to a degree which prevented her from reading fine print. Coincident with the onset of blurring of vision (i. e., in November 1932) vague, dull headaches occurred from time to time. While the headaches grew more severe as time went on, they were never an important part of the clinical picture. Early in 1933 the patient noticed occasionally a sensation of numbness, tingling and aching in the finger tips of both hands. At about the same time there was diminution in the sense of smell on the right. On careful questioning it appeared that attacks of nausea without vomiting developed as early as 1931 but were not severe enough for her to attach any particular significance to them. In the same year the menses became scanty, and since five months before admission to the hospital there had

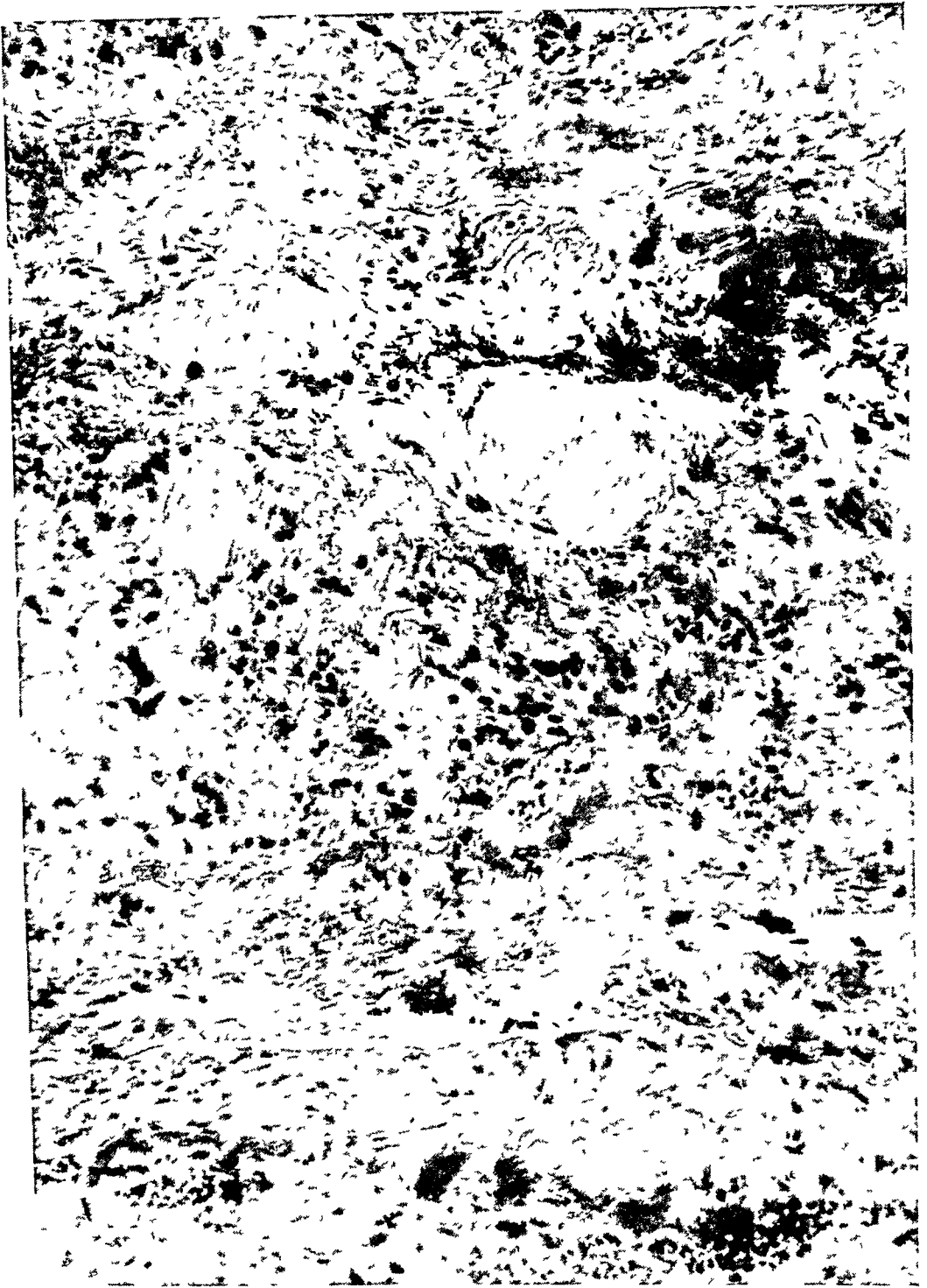


Fig 7 (case 2) —Photomicrograph of a section through the motor root of the left trigeminal nerve. The individual nerve bundles are intact, though the tumor has separated them from one another.



been no flow. Just before the menses ceased and at intervals after that there were occasions when the patient felt very warm and when blood seemed to rush to the head.

*Examination*—The patient was alert, well developed and in no acute distress. The skin was normal in texture, the hair of the body followed the distribution expected in women. The weight of 57 kilograms in comparison with a height of 147 cm gave a clinical impression of slight adiposity. The extra fat was accumulated over the abdomen and hips for the most part. The pupils reacted to light equally, and there were no abnormalities in the extraocular movements. The visual acuity in the left eye was 20/40 and that in the right eye 20/50. Charts of the fields of vision showed complete bitemporal hemianopia. On examination of the fundus there was slight fibrosis in the lamina cribrosa. Slight tortuosity was noted in the veins of both fundi, but there was no choking of the optic disk. At this time no tumor could be seen in the nasal cavities. A complete physical examination, including neurologic study, showed no further abnormalities.

*Roentgen Observations*—The cranial vault was of average thickness. The pituitary fossa was definitely enlarged and appeared expanded. There was considerable destruction of bone, particularly on the left, this also involved the dorsum sellae and the medial end of the left petrous ridge (fig 8). The sphenoid and petrous ridges were otherwise normal. The sphenoid sinus was cloudy. No definite soft tissue mass could be visualized in the nasopharynx. The impression was that of a rapidly growing, destructive tumor of the pituitary body.

*Operation*—The region of the pituitary body was exposed by turning down a right transfrontal bone flap. The greatly enlarged right optic nerve was dislocated upward and laterally by a large tumor. The operator then made a small opening in the tumor by way of the diaphragma sellae, this was followed by an outpouring of neoplasm so soft that it seemed more like a thick fluid than tissue. An endeavor was made to obtain more satisfactory specimens for histologic study with the hypophysial rongeur than could be secured with the hypophysial spoon. The former instrument was accordingly introduced into the center of the tumor and a fragment of tissue gently removed. Immediately the whole field was flooded with bright red blood. The operator at once realized that he had entered the internal carotid artery, which had been involved by the tumor to such an extent that very slight pressure had opened it. The hemorrhage was controlled, at first with gauze, and then with a large piece of muscle. Closure was accomplished successfully. The patient remained in a precarious state and died seven hours after closure had been completed.

*Necropsy*—The anatomic diagnoses were malignant adenoma of the pituitary body, invading the sphenoid sinus and nasal cavities, hyperplasia of the thymus (18 Gm), miliary adenomas of the adrenals, hirsutism of the lips, focal cicatrization of the pancreas, leiomyoma of the uterus, *Ascaris lumbricoides* infestation of the terminal portion of the ileum, renal calculi, uric acid deposits in the kidneys.

When the bone flap was elevated, the region of the wound was found free from hemorrhage. The right frontal lobe was then raised from the orbital plate and the operative site brought into view. Near the left side of the tense hemorrhagic tumor the incision made at operation was visible. The reddish soft tissue of the neoplasm pouted through the opening, but there was no accumulation of

blood in this area beyond slight staining of all the tissues at the base of the brain. The upward growth of the tumor had so distorted the optic nerves that both of them at first passed posteriorly and superiorly after leaving the brain substance. They then took a long course forward to each orbit. The left internal carotid artery was displaced from its usual position so as to lie outside the capsule of the tumor, near the operative opening in it. Three millimeters posterior to the incision the wall of the left internal carotid artery was irregular and traumatized. Proximal to the point of injury the artery was distended with a thrombus of recent origin. The right internal carotid artery and cavernous sinus were



Fig 8 (case 3) —Roentgenograms showing expansion of the sella turcica with considerable destruction of bone. The cloudiness of the sphenoid sinus proved to be due to a mass of tumor in that location.

included within the tumor, along with the adjacent nerves. There was no evidence of thrombosis or laceration of any of these vessels.

As for the tumor itself, it was situated in the greatly expanded sella turcica and was divided into two approximately equal portions by the sagittal plane of the brain. The main mass of the neoplasm measured 32 mm in the coronal plane, 18 mm in lateral extent, and 22 mm in the greatest anteroposterior plane. The soft, homogeneous, reddish purple tumor appeared entirely encapsulated except in the region of the floor of the sella turcica as will be described. Superiorly the tumor was limited by the compressed floor of the third ventricle, anteriorly,

by the optic chiasm and the anterior commissure, posteriorly, by its distended capsule, the remnants of the thinned dorsum sellae and the posterior commissure (fig 9)



Fig 9 (case 3)—Median sagittal section of the brain showing the extent of the tumor and its relations

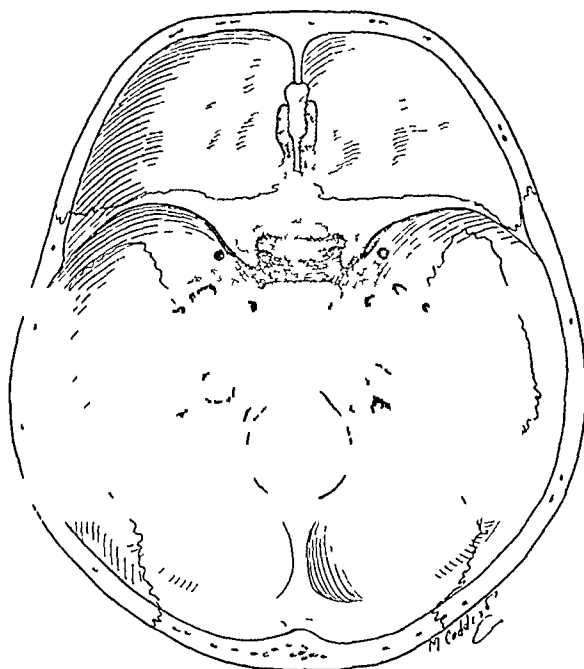


Fig 10 (case 3)—Diagram of the base of the skull to show the extent of bone erosion (stippled area)

There were widespread erosion of bone and extension of tumor at the base of the skull adjacent to the sella turcica (fig 10) Both anterior clinoid processes were eroded, and the dorsum sellae was largely destroyed. A perforation was

present in the floor of the sella turcica, in the posterior portion, and the tumor extended through it into the sphenoid sinus. There was a narrow partial septum dividing the sinus into two compartments, each of which was filled with soft tumor tissue in direct continuity with the main mass of neoplasm. The floor of the sinus was defective anteriorly on each side, with the result that a probe could be passed downward into the superior portion of each nasal cavity. Inspection from below showed that there were two defects, each 2 mm in diameter, on the left side of the nasal septum above the superior turbinate. No tumor tissue was found within the left nasal cavity. In the right nasal cavity a nodule of

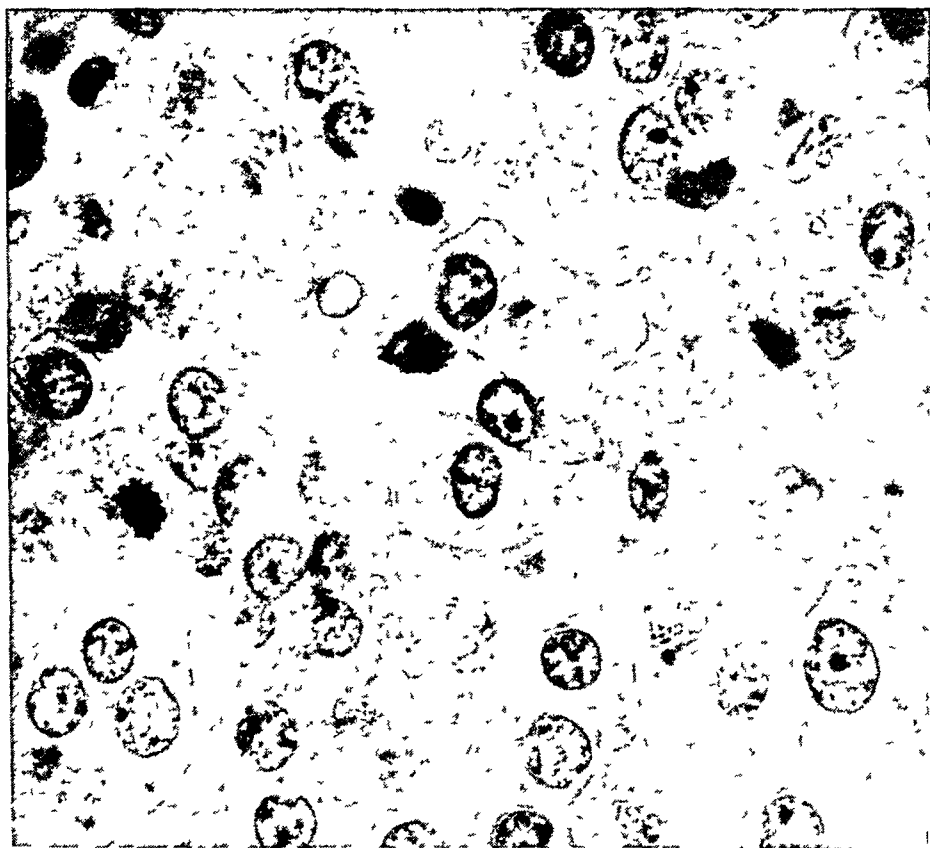


Fig 11 (case 3) —Photomicrograph to show the morphologic variation in the cells. Note the cell with the vacuoles at the center. This appears to be an earlier stage of the vacuolation shown in figure 12. There is a mitosis at the left. Hematoxylin and eosin,  $\times 900$ .

neoplasm extended for a distance of 8 mm. All the tumor tissue was directly continuous, and no metastases were found in the skull or elsewhere in the body.

After fixation in solution of formaldehyde U S P, the brain was cut in the midline anteroposteriorly (fig 9). The tumor was soft and varied in color from reddish purple to pale gray. Along the anterior aspect of the neoplasm there was a thin gray line, which proved to be a portion of normal pituitary tissue. The floor of the third ventricle was thinned from compression by the tumor, and there were several defects in it in the region of the intermediate mass and others near the posterior commissure. The third ventricle itself was not dilated.



Fig 12 (case 3)—Photomicrograph to show details of cell structure. A vacuolated cell of the type discussed in the text is shown just above the center of the photograph. There is a mitosis at the lower right. Hematoxylin and eosin,  $\times 1,700$ .

The pineal gland was situated 10 mm posterior to its usual site but otherwise was not altered. The aqueduct of Sylvius measured 1 to 2 mm in diameter. The fourth ventricle was normal.

*Microscopic Examination*—The material available for histologic study had been obtained (1) at the operation on the nose in Turkey, (2) at the operation in this hospital and (3) at necropsy. Sections from all three lots of material were prepared and stained by the methods listed in the report of case 1. The sections of material obtained at the second operation and of that obtained at

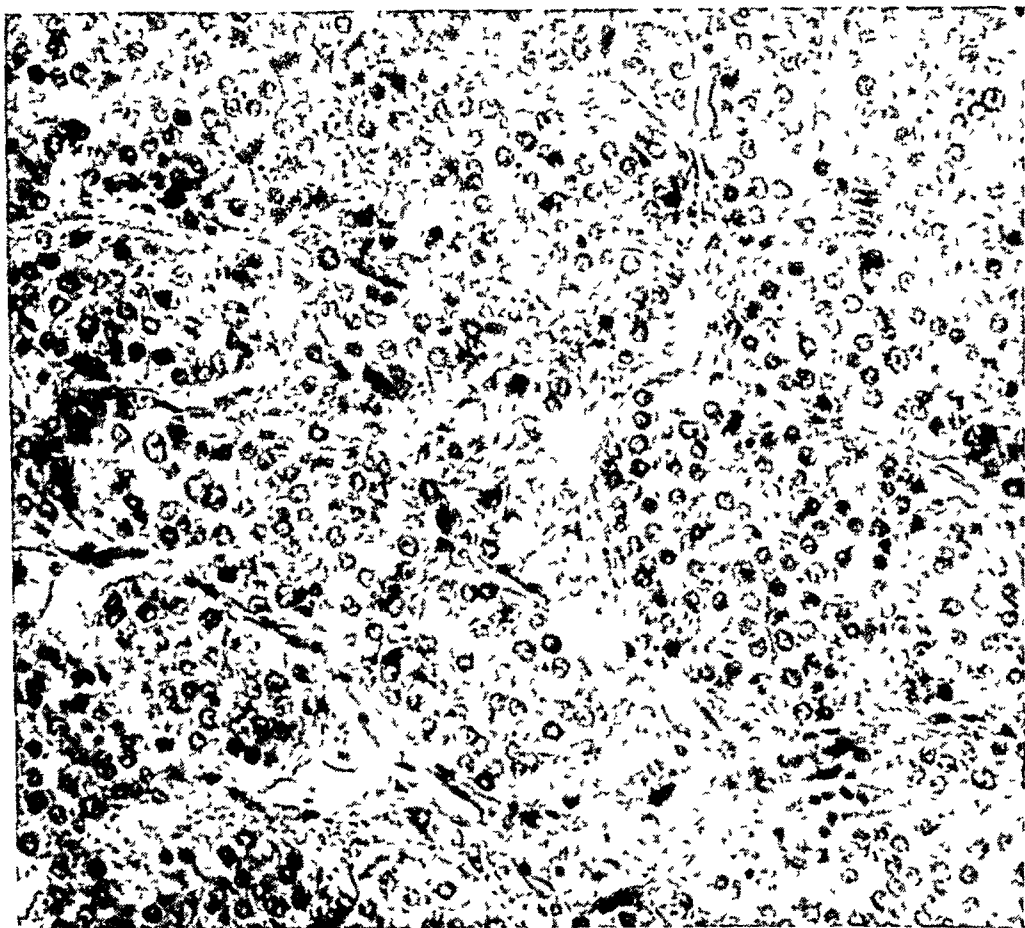


Fig 13 (case 3)—Photomicrograph of the tumor showing the formation of clusters of cells separated by a coarse collagenous connective tissue, quite different from that of the normal pars anterior. Hematoxylin and eosin,  $\times 300$ .

necropsy presented no essential differences and are described together. The sections of the material from the nose showed certain variations, which are discussed separately.

The nucleus of the type cell was round and contained a moderate amount of chromatin. The cytoplasm took a light stain, which appeared uniform until it was studied with the highest power microscopic objective, then fine granules, neutral in staining reaction, could be resolved. The cell wall was more distinct than that of the usual chromophobe cell. The cytoplasm of occasional cells contained clear vacuoles (fig 11). These usually occurred singly, though in some

cells the cytoplasm was filled with vacuoles (fig 12). The cells with vacuolated cytoplasm were scattered rather uniformly throughout the tumor and were not associated with areas of necrosis. Even when vacuoles were not present, there was considerable variation in cell size and shape. Mitotic figures were present in moderate numbers throughout (figs 11 and 12). No chromophilic cells were seen.

Although the tumor was richly cellular, there was in many areas a tendency toward arrangement of tumor cells in small masses (figs 13 and 14). Among the cell masses were strands of connective tissue, loose in texture and different in

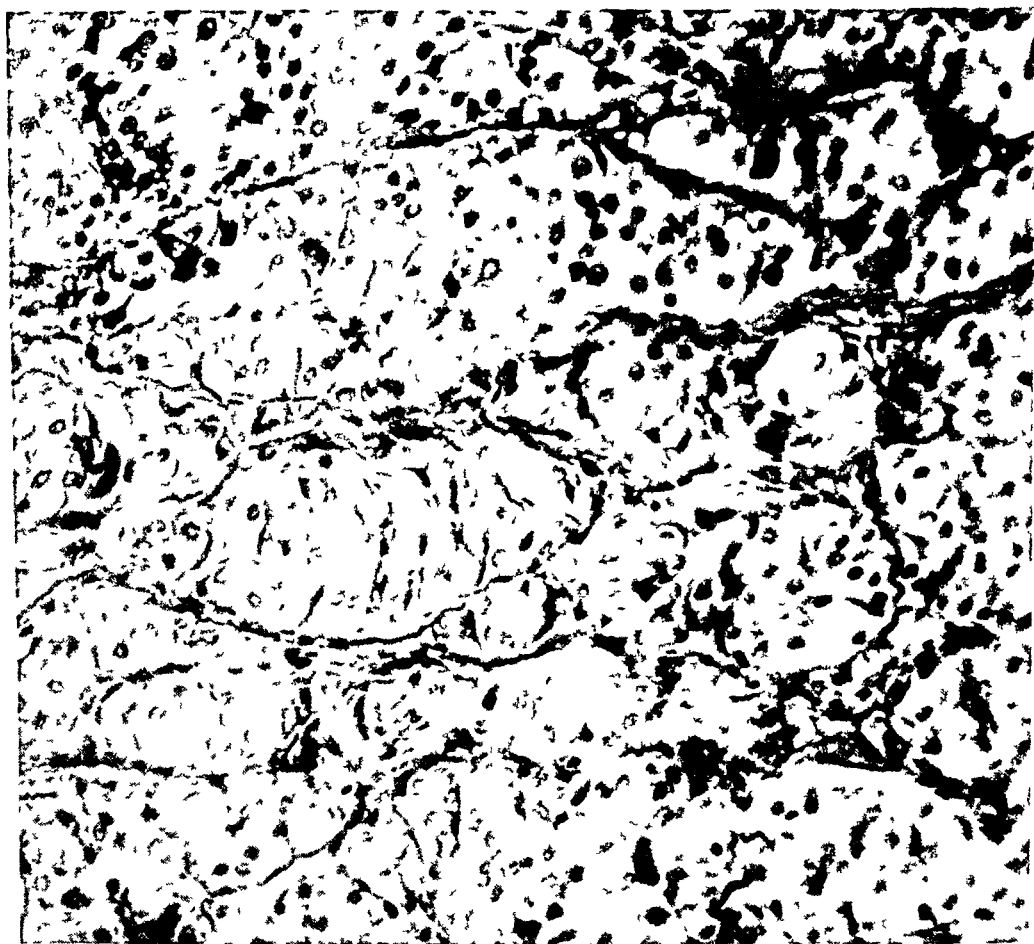


Fig 14 (case 3) —Photomicrograph of an area in which there were numerous vacuolated cells (See figs 11, 12 and 15). Note the coarse connective tissue separating the closely packed groups of cells from one another. Hematoxylin and eosin,  $\times 300$ .

character from that of the normal pituitary body. Only seldom were the cell groups penetrated even by the smallest collagen fibers (reticulum). This stroma did not resemble dural connective tissue and was definitely not old connective tissue included by the extending tumor. Moderate numbers of small capillaries were scattered through the tumor, but they also seldom penetrated into the cell masses. The dural margin was rather uniform along the tumor edge, no small clusters of cells extended into it. One large vessel in this region showed marked

endothelial proliferation, but elsewhere the walls of vessels were normal. There was no intravascular growth of neoplastic tissue. Extensive necrosis and some hemorrhage were found in the tumor, part of the latter being postoperative.

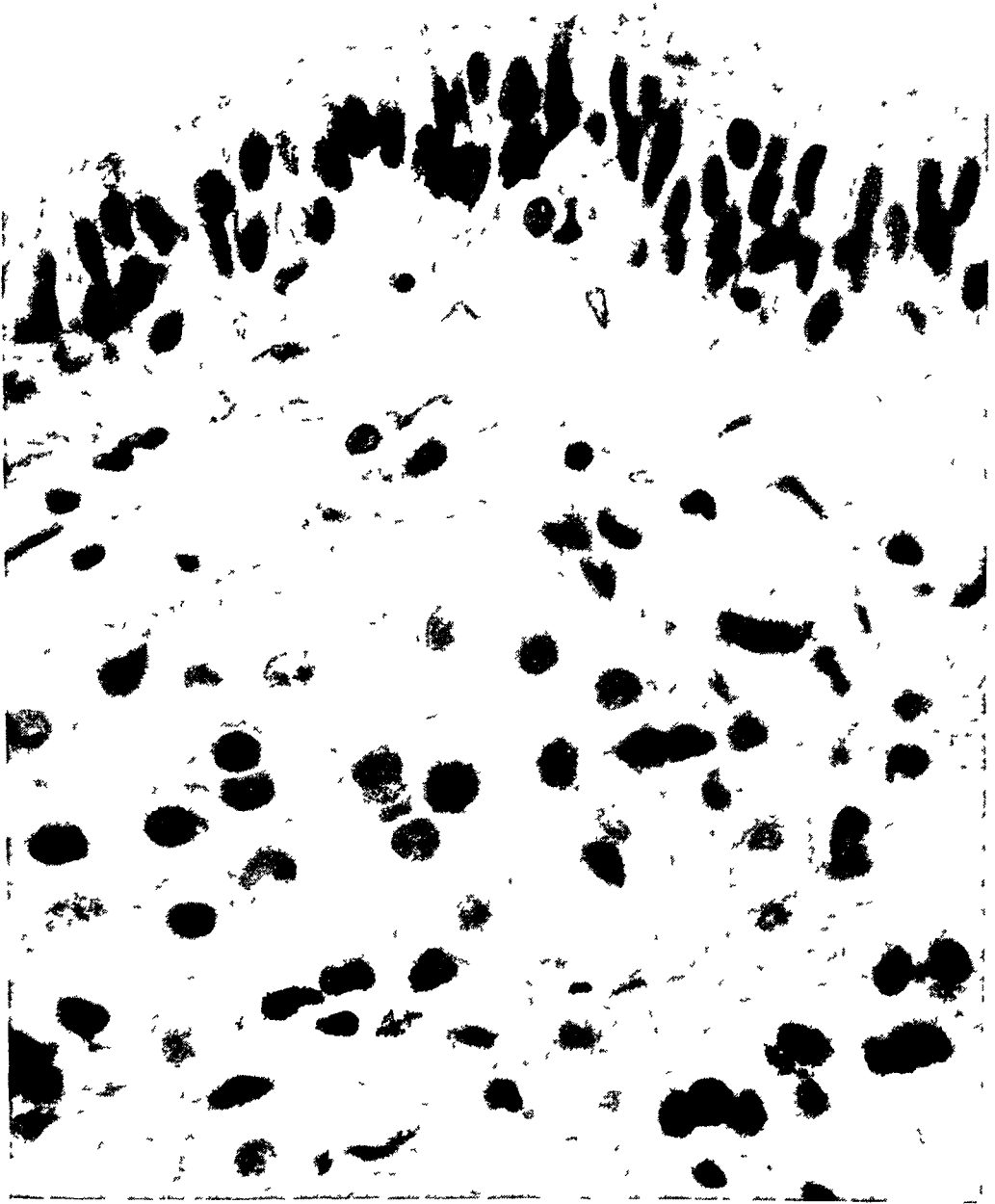


Fig 15 (case 3) —The edge of the intranasal projection of the tumor is shown. The nasal mucosa is intact. Just beneath it are closely packed tumor cells, most of them vacuolated. Hematoxylin and eosin,  $\times 900$ .

The material removed from the nose in Turkey was somewhat different in appearance. The superficial surface was covered by ciliated epithelium, which was intact in most places (fig 15) but showed here and there small ulcerated



areas. In the latter areas the edge of the tumor extended to the surface, but in other places it was separated from the normal mucous membrane by a narrow band of collagenous connective tissue. The tumor cells were grouped in discrete masses, separated from one another by strands of connective tissue. This stroma

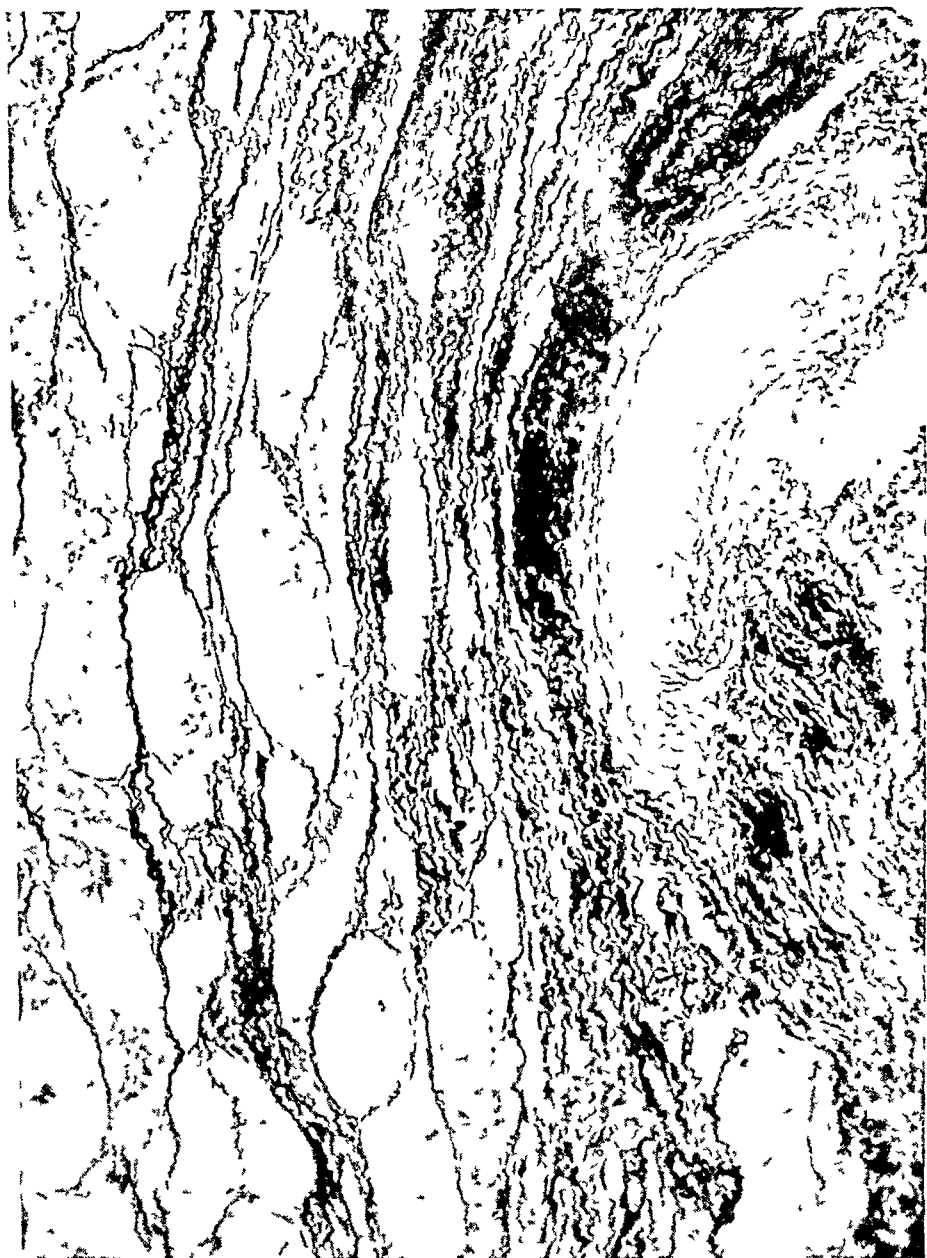


Fig 16 (case 3) —Photomicrograph to show the behavior of the stroma at the edge of the tumor. Foot's method for reticulum,  $\times 300$

was derived from the connective tissue of the nasopharynx. Figure 16 gives an indication of the basis for this statement, though the conclusion was reached only after study of many sections stained in various ways. The original connective tissue consisted of bundles of closely packed parallel wavy fibers, which stained

dark red with van Gieson's stain but were not impregnated by Foot's method for reticulum. The stroma of the tumor was more delicate in texture and was deeply impregnated by the silver method. In places where continuity between the two could be seen, the new connective tissue grew out of the old irregularly and extended in all directions from the parent tissue. The individual cells in the nasal extension were much like those of the center of the tumor, though the percentage of vacuolated cells was higher in the material from the nose. Mitoses were frequent in this portion. Unfortunately, none of the material was fixed suitably for glycogen stains, and the specimen was too old when this study was undertaken to permit the use of reliable fat stains. Therefore it was impossible to determine the content of the vacuolated cells by selective staining methods.

#### COMMENT

The foregoing pages contain descriptions of three unusual tumors arising from the chromophobe cells of the pituitary body. The patients would be exceptional among those suffering from pituitary tumors if only for the marked departure of their clinical course from that of patients with the syndromes usually caused by such lesions. The common characteristics which tend to set these tumors apart from the usual variety of chromophobe adenoma are their short history and the evidence of rapid extension into both the bone and the neighboring brain substance. A more detailed consideration of the gross and microscopic appearances of the three tumors and of certain tumors described in the literature points to the conclusion that they represent a small but distinct group—the malignant chromophobe adenomas of the pituitary body. They possess many of the criteria used by general pathologists in the diagnosis of malignancy in a tumor of any organ. Yet they do not metastasize if, indeed, the case of Dott, Percival Bailey, and Cushing<sup>2</sup> is excepted. This case is discussed later. What is a tumor to be called which is locally malignant but which rarely, if ever, metastasizes? We have chosen to use the term "malignant adenoma" rather than "adenocarcinoma," the only two apparent alternatives unless a term be coined. The use of the term "malignant adenoma" brings the classification of the tumors in line with that used by Cutler and Gross<sup>7</sup> for tumors of nerve sheath origin. They have called the entirely encapsulated lesion neurofibroma, the locally invasive tumor malignant neurofibroma and that producing widespread metastases neurofibrosarcoma. The malignant adenoma of the pituitary body and the malignant neurofibroma each represent neoplasms which are locally invasive but which do not produce distant metastases.

We return to the histologic characteristics of this group of tumors. In the type cell the cytoplasm is abundant, but its limits are often poorly defined, it is filled with minute granules, which stain very lightly and are not strongly acidophilic or basophilic. The nucleus is round or

<sup>7</sup> Cutler, E., and Gross, R. *Arch Surg* 33 733, 1936

oval, usually with one prominent nucleolus. These are all characteristics of the familiar chromophobe cells of the normal pars anterior and of the usual type of chromophobe adenoma. In the 3 cases discussed here, however, the individual cells diverged in certain respects from this pattern. Many cells were larger than normal chromophobe cells, while some were considerably smaller than the normal ones. On the whole, the tumor cells were closely packed in broad sheets and irregular masses. In case 3 there were vacuoles in the cytoplasm of many of the tumor cells in all parts of the neoplasm but especially in those in the intranasal portion (figs 11, 12, 14 and 15). Similar vacuoles were found in a few tumor cells in case 2 but not in case 1 or in the 81 cases of benign chromophobe adenoma of the pituitary body reported previously.<sup>6</sup> The vacuoles varied in size from small dioplets (as in the cell near the center of fig 11) to a size so large that several occupied almost the entire cytoplasm (fig 12). Vacuoles were not found within the nuclei. Studies have failed to demonstrate the exact nature of these cytoplasmic dioplets. Cells containing the vacuoles showed no evidence of fragmentation or nuclear disintegration, which makes it unlikely that the vacuoles were the result of cell degeneration. In all tumors of this series mitoses were seen in moderate numbers. In some chromophobe adenomas of the usual type, a few mitoses can be found after search. However, the mitoses in the three tumors to which this study is devoted were scattered through all parts of the tumors in greater numbers. There were, however, a few foci in which mitoses were especially numerous, the only characteristic which seemed to display significant variations in different parts of the tumors. Cell type, arrangement and stromal relationships retained that uniformity which has been illustrated in a previous paper.<sup>8</sup> In summary, then, the cells in all three tumors were derived from the chromophobe elements of the pars anterior but differed from them and from the cells of the usual chromophobe adenoma in variation in size, with a tendency to be larger than normal, in irregularity of arrangement and in the frequency of mitotic figures. These are cellular characteristics commonly regarded as suggesting rapid growth in any tumor.

The character of the stroma in the three tumors under consideration seemed even more definitely to indicate that they should be set apart from the usual chromophobe adenomas. The chromophobe cell is an epithelial cell. As such, it must be in close relation to a supporting and nutritive stroma. This, it should be borne in mind, is just as essential for the growth of neoplasms originating from epithelial cells as it is for normal development. Now general pathologists have long recognized that the stroma called forth in a malignant tumor of any organ differs from the normal stroma of that organ. Any structural peculiarity

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8 Schnitker, Cutler, Bailey and Vaughan,<sup>6</sup> figures 1 to 4

of arrangement of fibers peculiar to the stroma of the organ in which the tumor takes origin is suppressed or disappears entirely. As a result of these changes, the stromas of carcinomas arising in such diverse structures as the endometrium and the epithelium of the breast tend more nearly to resemble each other than to simulate the appearance of the original stromas. In each of the 3 tumors under discussion the stroma was more irregularly distributed, coarser and more refractile than that of the normal pars anterior or of the usual chromophobe adenoma. Figures 13 and 14 show the dense connective tissue stroma and the tendency for it to separate the tumor cell masses from one another. Particular care was taken to be certain that this change in character of the stroma was present in various parts of the neoplasm and to exclude the possibility that the coarse, refractile fibers could have been a part of the connective tissue of the dura included in the expanding tumor. At the edge of the neoplasm in cases 2 and 3 there was definite evidence that the stroma was derived from the connective tissue of the organs infiltrated by the tumor and that it was not carried forward by the tumor. The power to stimulate the growth of connective tissue in a region distant from the site of origin of a tumor is an indication of malignancy. The edge of the neoplasm in case 1 could not be studied because only biopsy material was available.

There is little of special note in the arrangement and appearance of the blood vessels in malignant chromophobe adenomas. They are very vascular tumors, but so are the benign chromophobe adenomas. There is no special relationship of cells to blood vessels as in the sinusoidal type of benign adenoma.<sup>6</sup> In this material there was no instance of intra-vascular growth of tumor. Though the tumors were highly vascular, there were numerous large areas of necrosis. The necrotic areas showed complete destruction of tumor tissue, often with evidence of old hemorrhage as well as that incident to the surgical procedure.

It may be seen from this discussion that the segregation of malignant from nonmalignant chromophobe adenomas has been made independent of the size of the neoplasm. On the other hand, it is to be expected that the more rapid and infiltrative the manner of growth, the larger the tumor will be by the time the patient presents himself at the clinic. This is borne out in the reasons given by the patients for consulting a physician. In case 1 the presenting symptom was difficulty in vision, just as it is in most cases of benign chromophobe adenoma. In case 2 the patient was first troubled by headache and some difficulty in vision, but neither were severe enough to make him come to the clinic, he waited until he had an uncinate seizure. In the third case the first symptom which the highly intelligent patient regarded as significant was nasal obstruction. Thus attention was first directed in 2 of 3 cases toward a mass of tumor which was the result of extension of the growth

beyond the region of the sella turcica—in the first instance, into the temporal lobe, and in the second, into the nasopharynx

The infiltrative character of malignant chromophobe adenoma is well indicated by the extent of destruction of the skull. Erosion of the sella turcica is common with all pituitary tumors except the smallest, and many of the neoplasms arising from chromophobe cells which we classify as benign are associated with considerable involvement of neighboring portions of the skull. In each of the 3 patients of this group, however, there was wide destruction of the skull bones, which seemed to offer little resistance to the tumor (figs 5 and 10). Such free invasion allows the tumor to spread out more readily than a tumor of the benign type. Accordingly, the symptoms referable to involvement of the sellar region per se are apt to develop later and to be less striking when the lesion is malignant than when it is benign.

The roentgenograms of patients with malignant chromophobe adenoma sometimes show clouding of the sphenoid sinus, indicative of extension of the tumor there, as in case 3. This and the destructive character of the sellar lesion are valuable aids in the preoperative differentiation of the malignant from the benign adenoma of chromophobe type.

It is evident that a tumor of the pituitary body which so widely invades the sphenoid bone and neighboring parts of the skull must come into direct relation with important vessels and nerves. The optic tracts are usually pushed upward by an expanding pituitary tumor of any type. This may be somewhat less noticeable in some patients with malignant chromophobe adenoma because of the freer extension of such a tumor into the skull bones and surrounding structures (for example, case 2). The tumor also comes in close contact with the internal carotid artery and the cavernous sinus. The benign chromophobe adenoma tends usually, but not always,<sup>2</sup> to push aside these important structures, while the malignant one may be expected more frequently to incorporate these vessels within the advancing neoplasm. This was well shown in the postmortem material in case 2, in which the tumor cells extended into the adventitia of the internal carotid artery but did not involve its media. In case 3 this sort of extension led to a situation which precipitated an operative fatality. While there is no instance in this series, further growth in this location might well result in occlusion of the internal carotid artery and consequent encephalomalacia, especially if the circle of Willis was compressed at the same time. Cranial nerves of the region about the sella turcica other than the second may be involved by the tumor in such a way that they become ensheathed by the neoplasm. The continuity of individual nerve fibers is usually preserved (fig 7), but further involvement of this kind serves to explain the cranial palsies

which occasionally accompany a tumor of this type. The semilunar ganglion was invaded by the tumor in case 2. Its architecture was partly disrupted, but the nerve elements showed little, if any, necrosis.

When the malignant adenoma grows upward, it has free access to the temporal lobe. Here the tumor may form a secondary mass larger than the growth in the region of the sella turcica. It is important to bear in mind that the two masses are connected by a bridge of neoplasm and that the involvement of the temporal lobe is by extension and not by metastasis. The tumor bridge in case 2 is illustrated in figure 6. The involvement of the temporal lobe brings on a change in symptoms. As pointed out by Spiller,<sup>9</sup> careful neurologic study of patients with this type of lesion enables one to predict the extent and relationships of the tumor with great accuracy. He pointed out that the well known uncinate syndrome is the result of a lesion causing irritation and, furthermore, that destruction of the uncinate region leads to homolateral anosmia and agusia, progressive paralysis of the opposite side of the body and other symptoms, depending on the extent of the lesion. The importance of recognizing the possibility of extension into the temporal lobe is emphasized by the fact that the symptoms indicative of involvement of the uncinate region may be more striking than those of the original tumor. In such instances as case 2, the discrepancy between the symptoms caused by the primary growth and those caused by the temporal extension has led to operation directed toward the extension and not toward the primary growth.

This is not the place for a discussion of the differential diagnosis of chromophobe adenoma of the pituitary body as a whole. Reference is made to the papers of Biggart and Dott,<sup>10</sup> which contain a full discussion of this topic. There are a few special problems in differential diagnosis which must be discussed in this paper because they attain particular prominence in chromophobe adenoma of the malignant type. Methods for differentiating the benign from the malignant adenoma have been suggested in various places but may be summarized here. Ocular symptoms are frequently earlier and more severe in the presence of a benign than in that of a malignant adenoma because of the tendency of the latter to spread more freely outside the confines of the sella turcica. Attention is frequently attracted first to a malignant chromophobe adenoma because of symptoms of its more distant extension, as nasal obstruction or an uncinate seizure. Further evidence of the presence of a malignant chromophobe adenoma may be secured from the roentgenogram, which shows destruction of bone with extension to the temporal and even the occipital bones rather than the more localized

<sup>9</sup> Spiller, W. *Arch Neurol & Psychiat* **16** 73, 1926.

<sup>10</sup> Biggart, J., and Dott, N. *Brit M J* **2** 1153 and 1206, 1936.

enlargement of the sella turcica with displacement of adjoining structures seen with benign chromophobe adenoma. A soft tissue mass in the sphenoid sinus or in the nasopharynx is, of course, indicative of the malignant type of adenoma. A ventriculogram may demonstrate the presence of a temporal tumor, which when combined with evidence of enlargement and erosion of the sella turcica is presumptive evidence of a malignant pituitary adenoma with extension to the temporal lobe. While the final decision must rest on histologic study, it should be possible to decide with a fair degree of accuracy whether a chromophobe adenoma of the pituitary body is benign or malignant before operation is undertaken.

There is one type of tumor involving the sellar region which is much more apt to be confused with the malignant than with the benign chromophobe adenoma, the chordoma. Both the malignant chromophobe adenoma and the chordoma cause extensive destruction of the sphenoid and occipital bones, and each may extend into the nasopharynx. The manner of involvement of the cranial nerves may be much the same in both cases. However, the chordoma does not usually give rise to hypopituitarism.<sup>11</sup>

Carcinoma arising in the lining of the sphenoid sinus and carcinoma having its origin in the epithelium of the nasopharynx must also be differentiated from malignant chromophobe adenoma. From either of these sites carcinoma in unusual cases may grow into the sella turcica and destroy the pituitary body wholly or in part. The tissue in the nasopharynx or in the sphenoid sinus may resemble grossly extensions of a tumor arising in the pituitary body. The resemblance between the carcinoma of the nasopharynx or the carcinoma of the sinus epithelium and the malignant pituitary adenoma is not great as a rule. Confusion arises when the examiner is seeking the origin of a large tumor involving the sella turcica, the sphenoid sinus and the nasopharynx. We have encountered 2 instances in which a tumor involving these three structures arose from epithelium of the respiratory tract. One of the patients was a man of 52 who suffered from loss of taste and smell, nosebleed and protrusion of the left eyeball. On examination he was found to have bitemporal hemianopia and choked disk. Roentgenograms revealed erosion of the left clinoid process and of much of the rest of the sphenoid bone and the left orbital plate. The tumor was demonstrated by biopsy to be a carcinoma arising in epithelium of the respiratory tract. The tumor unexpectedly showed a very satisfactory response to intensive roentgen therapy, and the patient is in good condition four years after the biopsy was made. The second patient (the following data are quoted from the records of the Albany Hospital by permis-

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11 Hass, G. Arch Neurol & Psychiat **32** 300, 1934

sion of Dr. A. W. Wright) was a youth of 17 who presented progressive emaciation and enlargement of the liver. At necropsy there were many discrete nodules of carcinoma in the liver, obviously metastatic. The only other tumor was a large mass involving the sphenoid bone and the pituitary body, the latter had been destroyed by it. The impression at the necropsy table was that the neoplasm arose in the pituitary body and metastasized to the liver. On microscopic examination, however, the tumor cells were found to be undifferentiated but bearing no resemblance to chromophobe cells. A careful search finally showed among them certain cells with cilia, which established the relationship to epithelium of the respiratory tract. We believe that the patient mentioned by Dott, Percival Bailey, and Cushing<sup>2</sup> as having a malignant chromophobe adenoma of the pituitary body which metastasized to the liver probably had a tumor similar to the one in the second of these cases. In their paper they specifically mentioned alveolar arrangements of cells resembling those of the thyroid gland. We are hesitant in accepting as malignant chromophobe adenoma tumors in which there are large distinct alveolar groupings of tumor cells. In such instances careful study of material which has been well fixed and stained will often reveal cuticular borders or cilia, either of which make it certain that the tumor does not arise from chromophobe cells. Squamous or transitional cell carcinoma may also arise in this region, either from the nasopharyngeal epithelium or from remnants of Rathke's pouch.

The differentiation of carcinoma originating in the sphenoid sinus or in the nasopharynx from malignant chromophobe adenoma is important in determining whether the pituitary tumor ever gives rise to distant metastases. Carcinoma of the types here enumerated often metastasizes to the cervical lymph nodes, lungs, liver and other organs. On the other hand, in neither of our cases of malignant chromophobe adenoma studied at autopsy were there distant metastases even though the primary tumor was large. A survey of the literature relating to malignant pituitary adenoma indicates that the only instance of such a tumor with metastases reported was that described by Dott, Percival Bailey, and Cushing,<sup>2</sup> which, we feel, should be excluded from the group. Malignant chromophobe adenoma may invade a large area directly, but distant metastases have yet to be demonstrated. We have not seen implantations within the cerebrospinal axis.

The treatment of a malignant chromophobe adenoma presents a number of difficult problems. Because of the situation of the neoplasm, a complete surgical extirpation is impossible. Partial removal of the growth, however, can be performed by the same technic as that used in dealing with a benign chromophobe adenoma. There are several reasons why partial removal of a malignant adenoma may be less satisfactory than that of a benign chromophobe adenoma. The wide extension of the



malignant adenoma through the skull provides a partial decompression of the sella turcica, hence, the improvement following sellar decompression is greater with the benign than with the malignant adenoma. Since the malignant adenoma grows more rapidly than the benign, any improvement resulting from partial removal might be expected to be more temporary. Furthermore, the tendency of the malignant adenoma to surround the large blood vessels about the sella turcica, such as the cavernous sinuses and the internal carotid arteries, makes serious damage to them a distinct possibility. Surgical exposure of the tumor may be necessary for diagnosis and should be done if careful neurologic study and roentgenograms still leave the nature of the lesion in doubt. It is sometimes possible to remove a portion of an intranasal extension of the growth for histologic study, as was done in case 3.

Roentgen therapy is an agent of definite value in the treatment of these tumors. Our technic and our results with benign chromophobe adenoma have been summarized elsewhere.<sup>6</sup> The material at our disposal does not permit any final statement as to the ultimate value of roentgen therapy in dealing with malignant chromophobe adenoma. However, the only patient in whom we have given it a thorough trial (case 1) has done so well that we are encouraged to try it in more cases of this type. When the diagnosis can be made with certainty from clinical and roentgenologic studies, we suggest irradiation without preliminary surgical procedures, reasoning by analogy from our results with benign chromophobe adenoma.<sup>6</sup> The problem of treatment is even more difficult when there is an extension of the tumor in the temporal lobe. Surgical attack on the mass in the temporal lobe is a possible palliative procedure (as in case 2). Roentgen therapy has been successful in case 1 so far in controlling symptoms which point to involvement of the temporal lobe.

A word of caution is necessary in regard to the treatment of an intranasal extension of a growth of this type. When radium is applied to the mass through the nose, the result is often a fatality, because of the uncontrolled necrosis and subsequent infection.

Malignant adenoma of the chromophobe cells of the pituitary body thus presents problems in treatment which are different in certain respects from those of benign chromophobe adenoma. It is, therefore, important to separate instances of the malignant type of adenoma into a distinct group. Only in this way can data be accumulated so as to lead to improvement in the diagnosis and treatment of this type.

#### SUMMARY

Three cases have been reported in which a malignant tumor arose from the chromophobe cells of the pars anterior of the pituitary body. These differed clinically from the usual case of benign chromophobe

adenoma in the history of rapid progression of symptoms and in the rapid extension of the tumor into the skull bones adjacent to the sella turcica, the neighboring brain substance and the nasopharynx. The early stages of the illness were dominated in the first case by ocular symptoms, in the second by uncinatc seizures and in the third by nasal obstruction.

Histologically, the tumors were characterized by arrangement of the tumor cells in broad sheets separated from one another by a stroma which was altered in character from that of the normal pars anterior and was in part derived from structures at the edge of the tumor, far from the sella turcica.

Such tumors should be set apart from the usual chromophobe adenomas and designated in a distinctive way. The term "malignant chromophobe adenoma" indicates that they are locally invasive and possess certain of the histologic characteristics of malignant tumors but do not metastasize either in the cerebrospinal axis or elsewhere in the body.

The malignant chromophobe adenoma presents certain special difficulties in differential diagnosis, especially in distinguishing it from chordoma and carcinoma of the sphenoid sinus or of the nasopharynx.

The tendency of the malignant chromophobe adenoma to include large blood vessels in the sellar region makes surgical approach to it especially perilous.

One of the 3 patients showed a very satisfactory response to biopsy followed by roentgen therapy, including the control of symptoms pointing to involvement of the temporal lobe. The other 2 patients, who were treated surgically, died.

# NEURONOPHAGIA IN THE HUMAN CEREBRAL CORTEX IN SENILITY AND IN PATHO- LOGIC CONDITIONS

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A study has been made of the cerebral cortex of man in order to determine whether a process of neuronophagia occurs there which is as well defined as that reported recently <sup>1a</sup> in the brain of the mouse as a result of starvation to the point of inanition, and in senility

An increase in satellitosis and a process of "neuronophagia" have been described by numerous workers with human tissue in a variety of pathologic conditions, but the process of actual ingestion of the neuron by the glial cells has not been described, nor are clear illustrations of this process available

The changes in the Purkinje cells of the cerebellum in the mouse from youth to senility have been reported in an earlier paper <sup>1b</sup> An attempt to compare the changes in the nerve cells of rodents and of man during the aging process was made in a study of human Purkinje cells in 34 autopsies on persons ranging in age from newborn to 83 years <sup>1c</sup> Briefly, in old age the Purkinje cells of the mouse and of man showed a decrease in the amount of Nissl material, a change from the "clear" type of nucleus to the "basophilic" type and a loss of the smoothness of contour of the cell body

Observations on the pyramidal cells of the cerebral cortex in the senile mouse <sup>1a d</sup> have shown a decrease in Nissl material, a change from the "clear" to the "basophilic" property of the nucleus in these cells, and instances of apparent neuronophagia, an actual ingestion of the nerve cell by the glial cells

It has been, however, the demonstration of active neuronophagia in the cerebral cortex of mice starved to inanition <sup>1a</sup> which has made it

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1 Andrew, W (a) *J Comp Neurol* **70** 413, 1939, (b) *Ztschr f Zellforsch u mikr Anat* **25** 583, 1936, (c) **28** 292, 1938, (d) *Am J Anat* **64** 351, 1939

possible to describe this process as one of lysis and ingestion of the nerve cell and further has enabled us to recognize the stages in the process in senile mice and in the present study on human material

#### MATERIALS AND METHODS

In the present investigation sections of human cerebral cortex and of cerebellum from 25 persons have been studied. The causes of death of these persons were varied, but it has been hoped that differences due to the specific pathologic condition of each person at the time of death would be overridden by the use of tissues from a large number of persons. Previous work with the Purkinje cells had tended to show also that the age of the subject is usually a more important factor in determining the appearance of the nerve cells than is the particular pathologic condition causing, or contributing to cause, death.

An attempt was made to avoid grossly abnormal areas, and when practical the right cerebral hemisphere was used, in order to insure uniformity. In all but a few of the cases two sections were taken from the cerebral cortex—one from the motor area and the other from the occipital lobe. The specimens of cerebellum were taken from the lateral hemispheres. All material was fixed in solution of formaldehyde U S P (1:10), usually within three to seven hours after death. The tissue was then dehydrated in the alcohols, cleared in xylene, infiltrated, and embedded in paraffin blocks. In all cases a detailed gross and microscopic examination was made of the remaining viscera.

The blocks of tissue from the frontal cortex, occipital cortex and cerebellar cortex were sectioned at 8 microns and mounted on slides. The sections from 4 persons, usually of widely varying ages, were carried through the process of staining in the same glass slide tray, constituting a "series," in which a direct comparison as to staining qualities of the cells is readily made. All material was stained in a 15 per cent aqueous solution of cresyl violet for a period of seventy-five minutes.

Opinions as to the staining properties of the cytoplasm and nuclei, the shape of the cell body and the amount of satellitosis could be formed by a more or less superficial observation of the tissue under low power. It was felt, however, that a quantitative investigation was much to be desired.

For this purpose 100 cells from each region of each subject studied were carefully examined under the oil immersion lens ( $NA = 1.8$ ) of a Bausch and Lomb monobjective binocular microscope (magnification, 970). These cells were classified according to the amount of Nissl material in the cytoplasm, the staining quality of the nucleus and the number of satellites in contact with the cell body.

Stages in neuronophagia and the relative frequency of their appearance were noted and recorded.

The data on the degree of satellitosis were obtained by a count of the glial cells in contact with the body of each nerve cell. Both microglial and oligodendroglial cells were included in this count, and a differentiation was not attempted, as the relative roles of the two types are still obscure and their distinction with Nissl staining probably not accurate. Only cells in contact with or encroaching on the body of the nerve cell were counted. Those touching the dendrites or axons were not included.

The method of making an observation on the cerebral cortex consisted in focusing on a particular part of the section from the region being studied, classifying the large pyramidal cells in this field as to number of satellites, then

passing to the immediately adjacent region in a direction parallel to the surface of the gyrus, classifying the large pyramidal cells here, and continuing in this way until 100 cells had been classified. In the cerebellar cortex we simply followed the outline of the granular layer until 100 Purkinje cells had been observed and classified as to the amount of Nissl material and the staining properties of the nucleus.

## OBSERVATIONS

A study of the layers of the cerebral cortex reveals that definite neuronophagia is easily demonstrable in many of the specimens, and

TABLE 1—*Material of Investigation*

Subject	Age, Years	Race	Sex	Cause of Death
1	7	Negro	♂	Acute rheumatic fever
2	12	White	♂	Anterior poliomyelitis
3	19	Negro	♂	Transverse myelitis (fractured cervical vertebra)
4	20	Negro	♂	Sicklelemla
5	25	Negro	♂	Hemorrhagic meningitis following mapharsen
6	35	Negro	♂	Cerebral hemorrhage
7	38	Negro	♂	Hypertensive nephritis, uremia
8	38	Negro	♂	Tuberculoma of pons, tuberculous meningitis
9	40	Negro	♂	Hypertension and cerebral hemorrhage
10	43	Negro	♂	Acute pulmonary edema (pneumonic)
11	45	White	♂	Acute alcoholism
12	45	White	♂	Dementia paralytica
13	46	Negro	♂	Dementia paralytica
14	48	White	♂	Old cerebral infarct
15	49	Negro	♂	Traumatic injury of brain
16	55	Negro	♂	Cerebral hemorrhage
17	56	White	♂	Cerebral thrombosis
18	60	White	♂	Cerebrospinal syphilis
19	63	Negro	♂	Lymphogranulomatous stricture of rectum
20	65	White	♂	Cardiac failure
21	67	Negro	♂	Cerebral hemorrhage, hypertension
22	69	White	♂	Bacterial endocarditis with embolic infarcts of meninges, spleen and kidneys
23	70	Negro	♂	Coronary occlusion
24	75	Negro	♂	Hypertensive nephritis, uremia
25	86	Negro	♂	Traumatic pneumonia

\* The subjects from whom brain tissue was obtained at autopsy are numbered consecutively in the first column in the order of age at the time of death. In the second, third and fourth columns are given the age, race and sex of each subject, for instance, 7, Negro, ♂ indicates a 7 year old Negro male, 45, white, ♂, a 45 year old white male. In the fifth column is given the cause of death as listed in the autopsy charts.

with some searching, in the majority of them. It is in the layer of polymorphic cells, just below the layer of large pyramidal cells, that the most active phagocytosis is seen to occur.

In specimens 1 to 8, inclusive, we find neuronophagia infrequently and only after diligent search. In these specimens the process, when seen, is similar in all respects to the process as we have studied it in young mice which have been starved to inanition. It consists in an encroachment by the glial cell, usually a solitary cell, on the substance of the neuron, in a widening crescent, the body of the glial cell growing as the substance of the nerve cell disappears and is ingested. In specimen 8 it is possible to find more than one stage of the process in a single field among the polymorphic cells, under the oil immersion lens.

(fig 1 *B*) By proper focusing the actual outline of the clear cell body of the glial cell can be made out quite readily in these Nissl preparations

In man, as in the mouse, the nucleus of the nerve cell appears to be the most resistant cell organ and often remains, enclosed in a thin crescent of cytoplasm, when nearly all of the cell body has been phagocytosed

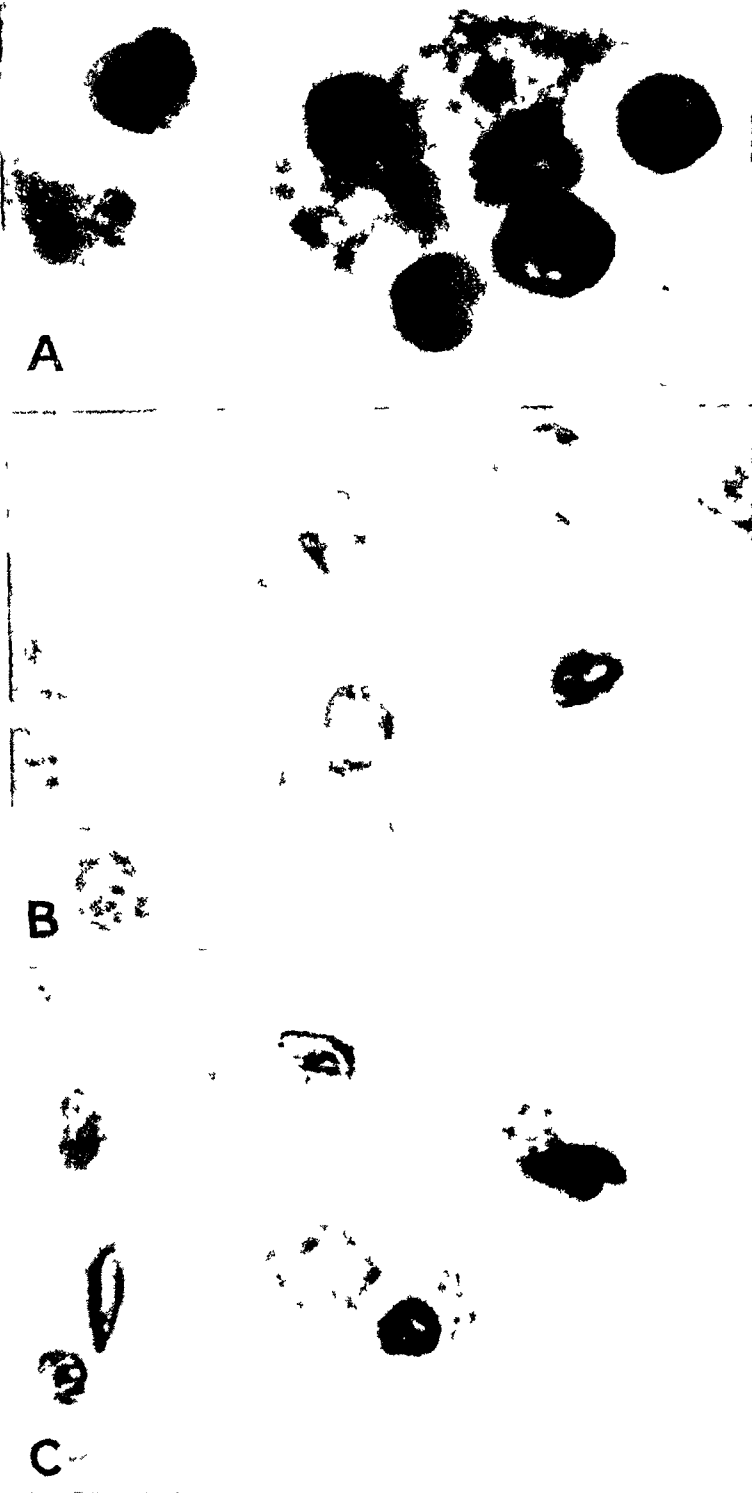
The process here, as in the mouse, appears to be essentially one of lysis followed by ingestion. However, in the end stages, as in the cell in the upper part of figure 1 *B*, there is sometimes at least an appearance as though the glial cell had engulfed the remaining formed portions of the neuron without lysis

In specimens 9 to 18, inclusive, neuronophagia is found considerably more frequently than in the preceding specimens. In these specimens the nerve cells more often are being phagocytosed by a group of glial cells, encroaching on the neuron from all sides. There are, however, still many instances in which only 1 or 2 glial cells are taking part in the process

In specimens 19 to 25, inclusive, neuronophagia is much more abundant than in the aforementioned specimens. In some of these specimens, particularly in 21 and 25, often several instances of neuronophagia may be seen in a single field in the layer of polymorphic cells, under the oil immersion lens. In these 7 specimens the neurons being phagocytosed almost invariably are being attacked by several glial cells, which sometimes form a ring about the cell body and at other times encroach on it predominantly from one side or the other. In figure 1 *A* a nerve cell is being attacked by such a group of glial cells, 3 of the cells destroying the cell body on one side, a single cell acting on the other

Among the nerve cells being phagocytosed in any of the age groups described there are large numbers which have by no means lost their typical structure. The nucleus and nucleolus may remain apparently intact even until the cell body has vanished almost completely, and a nerve cell in process of being destroyed frequently shows definite Nissl bodies

The chief differences in the amount of neuronophagia occurring in the layer of polymorphic cells and in the type occurring, i. e., whether 1 or 2 or a group of 3, 4 or 5 glial cells are active in the phagocytosis of a single neuron, are more closely correlated with age than with any of the other differences among the 25 subjects. These age differences resemble those seen in the mouse. In the brains of young healthy mice neuronophagia is not seen. When, however, neuronophagia is induced by starvation to the point of inanition, the process of ingestion of a whole neuron usually is accomplished by a single glial cell. In senile animals, adequately fed, neuronophagia can be found in many instances



EXPLANATION OF FIGURE 1

*A*, a nerve cell from the layer of polymorphic cells of the cerebral cortex of an 86 year old man, specimen 25, in process of being phagocytosed by four glial cells, the outlines of which can be seen clearly Nissl preparation,  $\times 2,500$

*B*, two nerve cells from the layer of polymorphic cells of the cerebral cortex of a 38 year old man who died of widespread tuberculosis (specimen 8) The cell above has been phagocytosed almost completely, and the large, "swollen" body of the glial cell can be seen The cell below has been only partially phagocytosed, the nucleus and nucleolus being still visible Nissl preparation,  $\times 1,000$

*C*, nerve cell from the polymorphic layer of the cerebral cortex of the same subject (specimen 8) The cell is partially phagocytosed by a glial cell, the clear cell body of which can be seen invading the cytoplasm of the neuron Nissl preparation,  $\times 1,000$

and is of the type in which several glial cells attack a single neuron. In starved senile animals this process is greatly accentuated, but in nearly all cases several glial cells attack a single nerve cell.

Neuronophagia was found to be far less active in the layers superficial to the layer of polymorphic cells. Satellitosis is present as a normal feature in the layer of large pyramidal cells but in those brains in which neuronophagia was very abundant, the degree of satellitosis, as judged by low power observation, was definitely increased. Instances of actual phagocytosis of the large pyramidal cells in such brains could

TABLE 2—Data on Large Pyramidal Cells of the Motor Cortex

Subject	Age, Years	100 Nerve Cells Grouped According to Number of Glial Satellites*						Total Number of Satellites per 100 Cells
		0	1	2	3	4	5	
1	7	74	19	6	1	0	0	34
2	12	Motor cortex not obtained						
3	19	75	18	5	2	0	0	34
4	20	69	24	6	1	0	0	39
5	25	73	20	5	2	0	0	36
6	35	64	30	4	2	0	0	44
7	38	72	18	10	0	0	0	38
8	38	74	18	2	1	1	0	35
9	40	65	25	5	4	0	1	52
10	43	63	19	11	7	0	0	62
11	45	50	31	12	4	3	0	79
12	45	76	16	7	1	0	0	33
13	46	60	28	11	1	0	0	53
14	48	Motor cortex not obtained						
15	49	73	18	5	4	0	0	40
16	55	64	30	4	2	0	0	44
17	56	Motor cortex not obtained						
18	60	78	16	4	2	0	0	30
19	63	78	28	11	1	0	0	26
20	65	61	23	8	2	1	0	54
21	67	58	24	13	5	0	0	65
22	69	61	29	8	2	0	0	51
23	70	58	26	11	4	1	0	64
24	75	46	27	16	6	4	1	98
25	86	63	26	9	2	0	0	50

\* Under the heading "100 Nerve Cells Grouped According to Number of Glial Satellites" there are found in the "0" column the number of nerve cells with no satellites, in the "1" column the number with one satellite, and so on.

be found, but were many times less frequent than among the polymorphic cells.

It was thought desirable to obtain some quantitative check on the degree of satellitosis. In tables 2 and 3 are given the data on the large pyramidal cells classified as to the number of glial satellites in contact with them. A study of the tables shows, in spite of the considerable degree of variation, that there is a marked increase in the average amount of satellitosis in the older persons as compared with the younger. Thus in the motor cortex (table 2) in the 7 persons under 40 years of age the average number of satellites found in contact with the bodies of 100 large pyramidal cells studied is 37. In the 8 persons



of ages from 40 to 60 years, inclusive, the average number of glial satellites is 49. In the 7 persons of ages 63 to 86 years, inclusive, the average number is 58.

In the occipital cortex the average number of satellites in persons under 40 years of age is 39, in those from 40 to 60 years, inclusive, it is 44, and in those from 63 to 86 years, inclusive, it is 57. It does not seem profitable to attempt to explain the individual differences in the degree of satellitosis among persons of the same age group, but we do see in both frontal and occipital cortex a definite increase in the degree

TABLE 3—Data on Large Pyramidal Cells of the Occipital Cortex

Subject	Age, Years	100 Nerve Cells Grouped According to Number of Glial Satellites*						Total Number of Satellites per 100 Cells
		0	1	2	3	4	5	
1	7	72	25	3	0	0	0	31
2	12	79	15	6	0	0	0	27
3	19	79	18	1	2	0	0	23
4	20	69	24	6	1	0	0	39
5	25	69	23	11	1	0	0	40
6	35	74	20	4	2	0	0	34
7	38	69	24	5	2	0	0	40
8	38	49	33	14	3	1	0	74
9	40	Occipital cortex not obtained						
10	43	60	29	8	3	0	0	54
11	45	Occipital cortex not obtained						
12	45	65	23	9	2	1	0	51
13	46	69	22	5	1	1	0	39
14	48	65	23	11	1	0	0	48
15	49	60	19	16	5	0	0	66
16	55	79	14	6	0	1	0	30
17	56	80	14	5	1	0	0	27
18	60	69	24	7	0	0	0	38
19	63	68	20	9	2	1	0	52
20	65	Occipital cortex not obtained						
21	67	46	30	17	5	2	0	87
22	69	78	21	0	1	0	0	24
23	70	63	24	7	5	1	0	57
24	75	Occipital cortex not obtained						
25	86	55	30	10	4	1	0	66

\* Under the heading "100 Nerve Cells Grouped According to Number of Glial Satellites" there are found in the "0" column the number of nerve cells with no satellites, in the "1" column the number with one satellite, and so on.

of satellitosis with advancing age in these persons. It seems probable that this increase in satellitosis is definitely related to the increase in neuronophagia.

The amounts of Nissl material in the large pyramidal cells of the cerebral cortex and in the Purkinje cells of the cerebellum show a very large degree of difference among persons even of very nearly the same age, as we might expect in dealing with such varied pathologic conditions. However, with a single very notable exception, the same persons show a relatively very large number of hypochromatic cells. The exception is the oldest specimen of all, that from an 86 year old Negro, in which the great majority of the nerve cells are chromatic. The body of this man was described as having been in excellent nutritional and



**A**

**B**

**C**

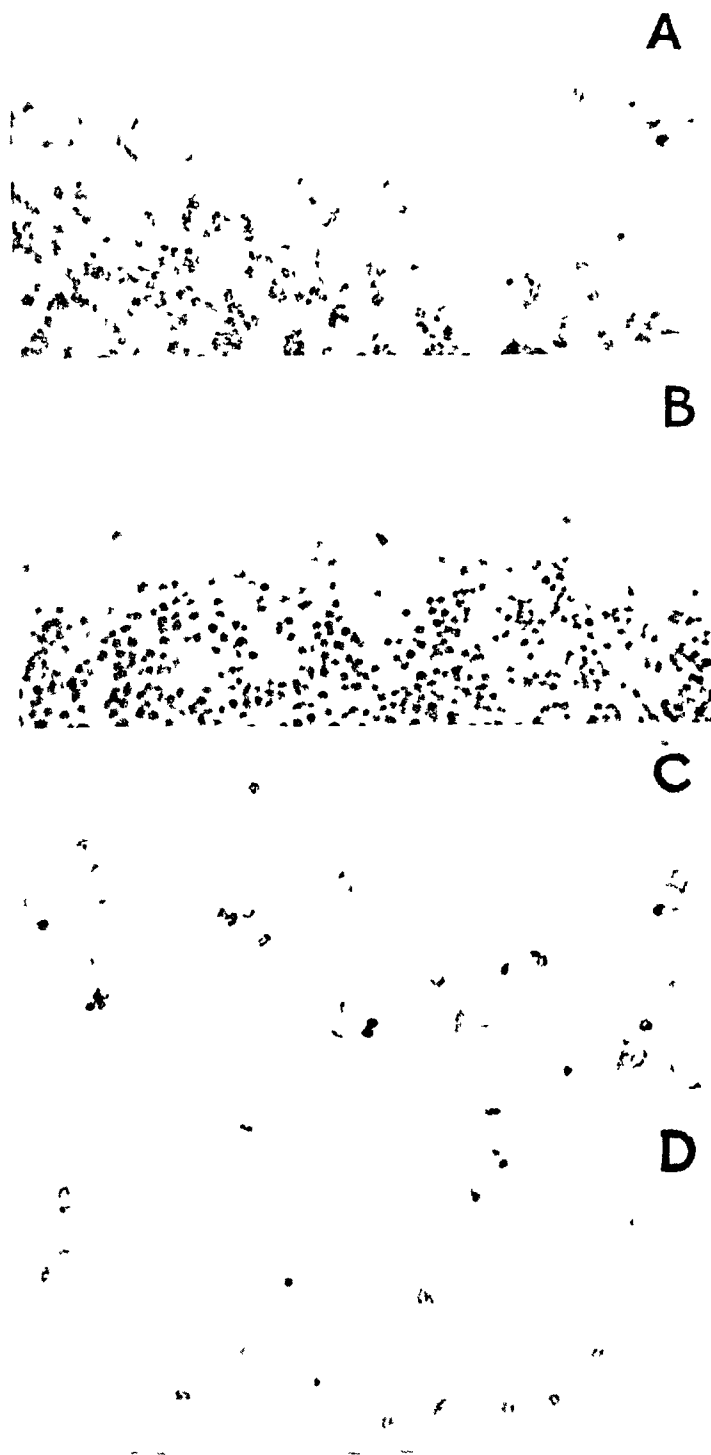
#### EXPLANATION OF FIGURE 2

*A, B and C are photomicrographs from Nissl preparations,  $\times 250$*

*A, layer of polymorphic cells of the cerebral cortex of a 19 year old man (specimen 3) The nerve cells are essentially normal. Satellitosis is not marked, and neuronophagia is rare*

*B, layer of polymorphic cells of the cerebral cortex of a 67 year old man (specimen 21) Satellitosis is marked, and neuronophagia is common*

*C, layer of polymorphic cells of the cerebral cortex of an 86 year old man (specimen 25) Satellitosis is common and neuronophagia abundant*



#### EXPLANATION OF FIGURE 3

*A, B, C and D* are photomicrographs from Nissl preparations,  $\times 250$

*A*, Purkinje cells of a 12 year old boy (specimen 2) The Nissl granules are abundant and the nuclei clear

*B*, Purkinje cells of a 75 year old woman (specimen 24) There is little or no Nissl material in these cells, and the nuclei are basophilic

*C*, layer of large pyramidal cells of a 19 year old man (specimen 3) The cells contain considerable Nissl material

*D*, layer of large pyramidal cells of a 75 year old woman (specimen 24) A large proportion of the cells are hypochromatic

muscular condition at the time that it came to autopsy. Whether this general condition may be the explanation of the condition of the nerve cells, we cannot say. Certainly it has been shown that it is possible for a senile person to have a majority of nerve cells with abundant Nissl material.

There is an increasingly larger number of nuclei with basophilic staining properties in the older persons. To this statement, our oldest subject is, however, again an exception.

In the majority of the subjects the relative numbers of chromatic and hypochromatic cells seen in the motor area, in the occipital cortex and in the cerebellum correspond fairly closely. Thus the condition of cells throughout the brain at the time of autopsy seems to be very similar in any given person.

No evidences of neuronophagia are seen in the cortex of the cerebellum nor is there any increased satellitosis about the Purkinje cells in senile persons.

#### COMMENT

The most important conclusions from the present study are that neuronophagia occurs in the cerebral cortex of man and that the process is essentially similar to that which is induced in the mouse by starvation. Neuronophagia has been found to some degree in the layer of polymorphic cells and to a lesser extent in the more superficial layers of the cortex in the majority of the pathologic specimens examined. The most abundant neuronophagia was found, however, in the senile brains.

We have been unable to find any earlier work in which a clear demonstration of the process of neuronophagia in the human cerebral cortex has been made. Glial proliferation in the senile brain has, however, been noted by several other workers. The question which has not been answered by the earlier workers appears to be what such glial proliferation indicates, whether it represents merely a substitution, the glia taking the place of the degenerating nerve cells, or whether the glial cells are taking an active part in the removal of these nerve cells, perhaps even in the destruction of living nerve cells.

Both the earlier and the more recent authors have noted glial proliferation in the senile brain, and some of them have spoken of an increased degree of satellitosis. Many of these authors, however, have concluded that this is purely a substitution or have not attempted to interpret its significance.

Recent papers, such as that by Cardona,<sup>2</sup> have reaffirmed the importance of glial changes as a criterion of pathologic changes in the human brain.

<sup>2</sup> Cardona, F. *Riv di pat nerv* 50 141, 1937.

Gellerstedt,<sup>3</sup> who made an extensive study of senile brains, apparently concurred with earlier workers who did not look on increased satellitosis as indicative of neuronophagia. He stated (p 332) *Diese "Phagozyten," besonders der tieferen Rindenschichten, sind von Saigo 1907 als eine senile Trabantenzellwucherung erkannt worden* (These "phagocytes," especially those of the deeper cortical layers, were recognized by Saigo in 1907 as a senile proliferation of satellite cells). He seems to have agreed with the earlier author that these cells probably are not phagocytes but simply satellites. Gellerstedt did find that the progressive changes in glial cells, rather than the regressive, are characteristic of the senium (ibid, p 401) *Die regressive Gliazellveränderung gehört nicht zum senilen Bilde* (The regressive changes in the glial cells do not belong to the senile picture).

Belezky<sup>4</sup> also found an increased number of mesoglia cells without processes and with few processes (oligodendroglia) both in normal senility and in senile psychoses.

Gellerstedt<sup>3</sup> found also a definite parallelism between the degree of glia proliferation and the degree of atrophy of the brain, even in specific localities in the brain. Nevertheless, he evidently thought of neuronophagia rather as a conception than as a demonstrable process. Thus he wrote (p 336) *Das Eindringen von Mikroglia in die Drüsen der Hirnrinde war natürlich auch in unserem Material kein seltenes Ereignis ebensowenig, auf das Gesamtmaterial bezogen, das Auftreten von "Neurono"- und Gliophagien, vor allem im Hirnstamm und Kleinhirnstamm* (The penetration of microglia into the drüsen of the cerebral cortex was of course likewise not a rare occurrence in our material, just as little, in reference to the whole material, as was the appearance of neuronophagia and gliophagia, especially in the brain stem and in the cerebellar medulla).

Muhlmann<sup>5</sup> made a study of the glia in the human brain at various ages, but did not speak of the occurrence of neuronophagia nor even of any markedly increased degree of satellitosis.

Einarson and Okkels<sup>6</sup> spoke of neuronophagia as occurring in the cerebral cortex and in other parts of the brain of a 93 year old woman but did not describe or figure the process in their paper.

Schukru-Aksel,<sup>7</sup> in his study of the brain of "the oldest man in the world," found great numbers of a new type of large glial cell in the

3 Gellerstedt, N. Upsala läkaref. förh. **38** 193, 1933.

4 Belezky, W. K., and Jermolenko, E. I. Virchows Arch. f. path. Anat. **291** 607, 1933.

5 Muhlmann, M., and Mutalimow, B. Anat. Anz. **80** 218, 1935.

6 Einarson, L., and Okkels, H. Ann. d'Anat. path. **13** 557, 1936.

7 Schukru-Aksel, J. Arch. f. Psychiat. **106** 260, 1937.

cerebral cortex, basal ganglions, mesencephalon and dentate nucleus. He wrote (p 263) *Merkwürdig ist aber das Hervortreten eigenartiger Glakerne, die an Pseudosklerosezellen erinnern. Diese Glakerne sind diesmal so gross wie die normalen* (Noteworthy, however, is the emergence of peculiar glial nuclei, which recall pseudosclerosis cells. These glial nuclei are three times as large as the normal ones). He did not describe these as showing a tendency toward satellitosis nor did he describe an increase in satellitosis by the ordinary, smaller glial cells except in certain regions. He did say of the hypothalamus (p 265) *Man findet hier aber Triabantzellenansammlungen um die Nervenzellen herum* (One finds here, however, accumulations of satellite cells around the nerve cells).

Rothschild<sup>8</sup> said of the cases of senile psychoses which he studied (p 765) "Several cases were observed in which especially severe cell losses combined with considerable neuroglial proliferation produced outspoken disturbances of the cyto-architecture."

We see, then, that many authors have observed proliferation of glia in the senile brain, that some have noted increased satellitosis and that a few have spoken of neuronophagia as a function of the glial satellites. We feel that as a result of the present study we can without hesitation affirm that neuronophagia is occurring actively in the brains of the majority of senile persons. We believe also that the increase in satellitosis is definitely related to the process of phagocytosis. How long the destruction of a neuron in the senile brain may take, it is as yet impossible to say, but the indications are that the process may be a very slow one, certainly as compared with that in starved animals.<sup>1a</sup>

The process seen in these senile human brains appears to be essentially similar to that previously studied in the mouse. It consists in lysis and ingestion of the neuron by the glial cells, 1 to 5 or even more of these cells taking part in the phagocytosis of a single large neuron. Here, as in the mouse, the nucleus is usually the last of the cell parts to be ingested.

In man, as also in the mouse, the Purkinje cells appear to be wholly immune to any phagocytic action by the glial cells. We have yet to see a single instance of an attack on these cells. The only author whom we have found to speak of any increased degree of satellitosis or of phagocytosis of Purkinje cells is Marinesco,<sup>9</sup> who stated that such phenomena might be observed after injection of bile into the cerebellum.

The loss of Nissl material in the cells of both the cerebral cortex and the cerebellum was a feature of the senile brains studied in this investigation with the notable exception of the oldest of all, that of the

8 Rothschild, D. Am J Psychiat **93** 757, 1937

9 Marinesco, G. Ann d'anat path **7** 341, 1930

86 year old Negro Predominance of hypochromatic cells in the cerebral cortex of a woman dead of senility at 90 years of age was reported by Robertson and Orr<sup>10</sup> Salimbeny and G ry<sup>11</sup> reported increased acidophilia in the ganglion cells, especially in the Purkinje cells, of an 83 year old woman, together with irregularity of cell outline, atrophy, and degenerative changes in the nucleus Ellis,<sup>12</sup> in his quantitative study of the loss of Purkinje cells with advancing age, found degenerative changes in these cells in senility, including chromatolysis and atrophy

Einarson and Okkels<sup>6</sup> found that chromatolysis was a conspicuous feature in the brain of a 93 year old woman

Rothschild<sup>8</sup> in a detailed study of 24 cases of senile psychoses found many cells with pale, ill defined Nissl bodies, and in the more severe cases the Nissl material was altered to a dustlike material uniformly scattered throughout the cytoplasm He also found cell shadows, or "ghosts," to be common He said (p 782) "Equally severe alterations may be found in the brains of old persons of normal mentality "

The author of the most extensive work on changes in the brain in normal senile involution (Gellerstedt<sup>3</sup>) has, in spite of the findings by the other authors mentioned and by others not mentioned, taken the view that hypochromatism of large numbers of cells may not be a feature of normal senility, or that in life the normal senile brain may contain perhaps as many chromatic cells as the brain of a younger person and that the greater loss of Nissl material from the cells in senile persons may be due simply to the greater vulnerability of the cells in such persons to the damages wrought in the final stages just before death He said of the hypochromatic cells (p 270)

Solche waren nun in den verschiedensten Hirnregionen unsres Materials, und zwar in jedem Falle, zu sehen, gar nicht selten aber auch im jungeren und jungsten Kontrollmaterial gesunder wie kranker Individuen Verf ist also eher geneigt, ihnen Charakter als typische senile Ver nderung zu bestreiten und an praagonale Absterbeerscheinungen bei Ganglienzellen zu denken Dass sie jedoch im Senium so besonders h ufig sind, konnte mit der grosseren Vulnerabilit t alternder Zellen zusammenhangen (Such were to be seen in the most diverse cerebral regions of our material, and in every case, and not rarely also in the younger and youngest control material of healthy as well as of diseased individuals The author is therefore rather inclined to deny their character as typical senile changes and to think of them as preagonal manifestations of the dying process in ganglion cells That they are so frequent during senility would be due to the greater vulnerability of aging cells)

Gellerstedt examined a large amount of material, the brains of 50 persons over 65 years of age, prepared according to numerous technics,

10 Robertson and Orr J Ment Sc 44 729, 1898

11 Salimbeny, A T, and Gery, J Ann Inst Pasteur 26 577, 1912

12 Ellis, R S J Comp Neurol 32 1, 1920

and hence his conclusions should be worthy of serious consideration. We shall note, however, that he did find hypochromatic cells much more abundant in the senile material, and the question which he raised is: How did this greater abundance come about?

We cannot wholly agree with his answer to this question. We believe that the large number of hypochromatic cells is a characteristic of the average senile brain and not due to changes just before death. Support for this standpoint is derived from a comparative study of the brains of animals other than man, killed in a variety of ways. In all of the work of one of us (W. A.) on the mouse<sup>1a, b, d</sup> the loss of Nissl material from the nerve cells is a conspicuous feature of the brain of the senile animal. The great majority of the animals were killed by decapitation and the brain removed immediately and placed in fixative. The time from decapitation until the brain was in fixative was never more than three or four minutes, and it is thought unlikely that such striking loss of Nissl material as is seen would occur in so short a time. Other authors (Dolley,<sup>13</sup> on the dog, Inukai,<sup>14</sup> on the rat) have found increased numbers of hypochromatic cells in the brains of senile specimens among the lower animals.

We believe that the higher number of hypochromatic cells in the brains of older men and women speaks for the loss of Nissl material as a phenomenon of the aging process and as a gradual rather than a rapid or sudden process.

It does not seem surprising that there should be considerable variation in the degree of loss of Nissl material at any particular age when we consider the differences in other characteristics of persons in the same age group. An interesting example of this is seen in the report by Schukru-Aksel<sup>7</sup> on the brain of the "oldest man in the world," Zaro Aga, who, according to reliable data, must have been about 130 years of age at the time of his death. Schukru-Aksel wrote (p. 263) *Die Ganglienzellen enthalten ziemlich gut angeordnete Nissl-Schollen* (The ganglion cells contain rather well arranged Nissl material). The 86 year old man in our series showed, as we have said, abundant Nissl material. This is, however, we believe, an exception to a rule. In this man's brain neuronophagia was very common, as in the other senile brains.

The change in the staining properties of the nucleus which leads to a great decrease in the number of nuclei of clear type and an increase in those of basophilic type with increasing age is a phenomenon which had been noted previously in the mouse<sup>1a, b, d</sup> and in the Purkinje cells of man. We have been unable to find this phenomenon described

13 Dolley, D. H. J. M. Research **24** 309, 1911

14 Inukai, T. J. Comp. Neurol. **45** 1, 1928



clearly as an accompaniment of aging of the nerve cell in the papers of earlier workers, although there are some references to larger numbers of hyperchromatic nuclei in senile persons as in the paper by Einaison and Okkels<sup>6</sup> on the brain of an 83 year old woman

Butler,<sup>15</sup> in reporting on changes in depression of the nerve cell produced by long ether anesthesia, said (p 330) "In depression the nucleus contains increased amounts of nuclear materials, both chromatin and nucleolar substance, corresponding to the degree of depression, while the plasma is deficient in chromatin (Nissl substance) "

Among all the Purkinje cells examined in human senile brains no cells with double nuclei nor any showing amitotic divisions of the nucleus have been found, although such cells are encountered frequently in the brains of senile mice

We wish to call attention to the presence of pigment in almost 50 per cent of the Purkinje cells in the brain of one of the senile subjects, no 24 In this subject many of the large pyramidal cells also showed pigment Dolley<sup>16</sup> said concerning abnormal pigmentation in the nervous system (p 482) "It is a phenomenon of chronic depression, or depressant senility " Harms<sup>17</sup> found much pigment present in the ganglion cells from many parts of the nervous system of a woman 80 years of age

We have not, however, found pigmentation as a constant feature in the senile brains which we have examined

#### SUMMARY

Neuronophagia, a process of lysis and ingestion of the nerve cell, as demonstrated in the mouse,<sup>1a</sup> occurs in the human cerebral cortex in senility and in a number of pathologic conditions This process is most active in the layer of polymorphic cells

There is an increase in the degree of satellitosis about the large pyramidal cells with advancing age

A loss of Nissl material and an increase in the basophilic properties of the nuclei are found in most of the older persons and are believed to be natural phenomena of aging in man

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15 Butler, E E J M Research **34** 325, 1916

16 Dolley, D H J Comp Neurol **28** 465, 1917

17 Harms, J W Zool Anz **74** 249, 1927

## Case Reports

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### INSULIN HYPOGLYCEMIA AND GLYCOGENIC HEPATOMEGALY IN DIABETES MELLITUS

NATHAN B. FRIEDMAN, M.D., CHICAGO

Although the observation of increased amounts of glycogen in both the normally sized and the enlarged liver of the diabetic patient is an old one, it may be of value to record it again since the emphasis in recent studies has been on the deposition of fat in such livers.<sup>1</sup> The finding of large glycogen-laden livers has led some workers to the consideration of such a condition, even when it occurs in a patient with undoubted diabetes, as belonging to the category of glycogen storage disease (Von Gierke's disease).<sup>2</sup> Transitions from glycogen disease to diabetes are also recorded.<sup>2b</sup>

It is not the purpose of this paper to deny the existence of these transitional or mixed forms but to reemphasize the fact that glycogen storage may be a prominent feature of diabetic hepatomegaly. The cases to be reported illustrate hepatomegaly associated with insulin hypoglycemia in diabetic patients, and in these cases the hepatomegaly was due to storage of glycogen rather than to storage of fat.

#### REPORT OF CASES

CASE 1 — (This case is reported with the permission of Drs. Joseph Biennemann and William G. Hibbs.)

A 3½ year old boy was admitted to the Children's Memorial Hospital (service of Dr. Alvah Newcomb) in coma of one day's duration after three weeks' loss of weight, polydipsia and polyuria. He was comatose, dehydrated and breathing deeply and had an acetone breath. The temperature at its highest was 101 F. The blood pressure was 112 systolic and 66 diastolic. There were a few rales in the lungs and a barely palpable liver.

The urine showed sugar (4 plus) and acetone, and the blood sugar was 500 mg per hundred cubic centimeters.

He received insulin in large doses and, parenterally, dextrose solution, saline solution and lactate-Ringer solution (as devised by Dr. Hartman). During each eight hour period following admission he received about 1,000 cc of these fluids. The amount of insulin given in each of these eight hour periods was as follows: 180, 120, 330, 220 and 200 units (in the first three of these doses 120 units of protamine insulin are included). During the last eight hours of his life he received no insulin.

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From the Department of Pathology of the University of Chicago

1 (a) Warren, S. *The Pathology of Diabetes Mellitus*, Philadelphia, Lea & Febiger, 1938. (b) Marble, A., White, P., Bogan, I. K., and Smith, R. M. *Arch Int Med* **62**: 740, 1938. (c) Dragstedt, L. R., Van Prohaska, J., and Harms, H. P. *Am J Physiol* **117**: 175, 1936.

2 (a) Terplan, K., Vogel, S., and Hyde, E. *Arch Path* **26**: 599, 1938. (b) van Creveld, S. *Medicine* **18**: 1, 1939.

The blood sugar fell at one time to 196 mg and varied between this level and 500 mg per hundred cubic centimeters. The carbon dioxide-combining power remained low, between 14 and 21 volumes per cent. Sugar persisted in the urine (4 plus) but acetone later disappeared.

He remained in semicoma and had two episodes of convulsions with rigidity of the extremities. Twelve hours before death the blood sugar was 285 mg. Three hours later spastic opisthotonos developed, and cerebral damage was suspected. A spinal tap showed increased pressure, and the spinal fluid contained 222 mg of sugar per hundred cubic centimeters. Three hours later, however, the blood sugar was found to be 17 and one hour later 30 mg.

He died six hours later despite the fact that the administration of insulin had been discontinued and that five doses of 10 to 25 cc of 50 per cent dextrose solution had been given intravenously in addition to the continuous infusion.

*Pathologic Observations* (Dr W. Price Killingsworth)—The body weighed 26 pounds (11.5 Kg) and was 37 inches (94 cm) in length. The heart and kidneys appeared normal. The liver weighed 650 Gm (normal, 400 to 500 Gm<sup>3</sup>), was deep red and cut with resistance. The pancreas weighed 5 Gm (normal, 17 to 18 Gm<sup>4</sup>). The brain was congested and contained tiny focal meningeal hemorrhages.

*Microscopic Observations*—The heart and kidneys were essentially normal. The thyroid and adrenals showed no changes.

The liver cells were markedly vacuolated and clear (*A* in figure). They were laden with glycogen and showed relatively little fat (*B* in figure).

The pancreatic acinar tissue appeared normal, but the islets were scarce. In the body and head the islets were practically absent, and only in the tail did they approach the normal size and number. Their cellular structure appeared to be normal.

**CASE 2**—A 22 year old man was admitted to the University of Chicago Clinics (service of Dr. Louis Leiter) in March 1935, with hematuria and a painful mass in the right side of the abdomen.

Diabetes had been discovered at the age of 15, and he had been followed in the aforementioned clinics thereafter. At the outset he had required 25 units of insulin daily, but later took from 40 to 60 units. In February 1934 he had a preauricular carbuncle and required up to 80 units daily.

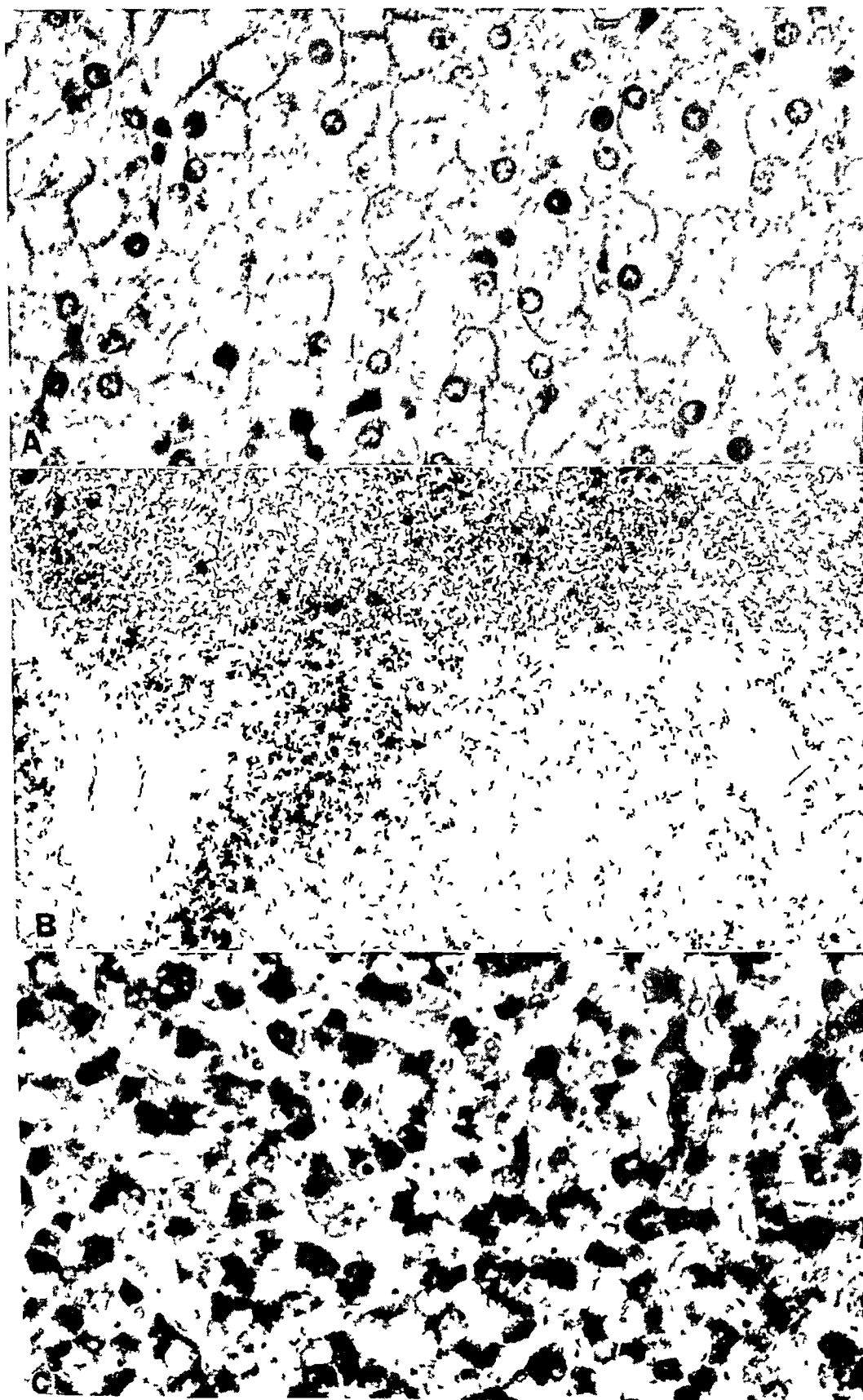
Laparotomy two days after his admission revealed a large pyonephrotic right kidney with an aberrant artery across the ureter, and the kidney was removed. Following this a septic temperature developed, ranging to 103 F, and blood cultures yielded *Staphylococcus aureus*. There was also bilateral draining otitis media.

His daily intake (orally and by vein) varied considerably, ranging from 50 to 250 Gm of carbohydrate, with a total caloric value of from 500 to 1,500. Although he was given from 40 to 120 units of insulin per day, the blood sugar remained high (201 to 470 mg per hundred cubic centimeters), and there was glycosuria (to 2 per cent).

On the fifth postoperative day he had episodes of irrationality and struggling. Meningitis was suspected, but lumbar puncture gave negative results. On the twelfth postoperative day his intake had included 130 Gm of carbohydrate (total calories, 600), and 60 units of insulin had been given. The urine contained no

3 Coppoletta, J. M., and Wolbach, S. B. *Am J Path* 9:55, 1933.

4 Gruber, G. B., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1929, vol. 5.



*A*, microscopic appearance of the liver in case 1 *B*, same liver stained for fat (sudan) *C*, liver in case 2 stained for glycogen (Best's carmine stain)

sugar. Then blurring of vision developed, with a positive Babinski sign, rigidity of the extremities and slowness of respiration with periods of apnea. On intravenous administration of 50 cc of 50 per cent dextrose solution he regained consciousness, his muscles relaxed, and respiration became normal, all within five minutes. Between two and three hours later, 20 additional units of insulin having been given, a similar seizure occurred and he died.

*Pathologic Observations* (Dr. Paul R. Cannon).—The body weighed 122 pounds (55 Kg) and was 66 inches (167.5 cm) in length. There were multiple abscesses in the remaining (left) kidney, thrombosis of the left renal vein, and abscesses in the myocardium, lungs and one epididymis. The heart weighed 280 Gm. The aorta was moderately atherosclerotic.

The liver weighed 3,100 Gm (normal, 1,500 to 1,700 Gm) and was smooth, pale and translucent. The pancreas weighed 47 Gm (normal, 70 to 90 Gm). The brain was edematous but revealed no meningitis.

*Microscopic Observations*.—There were some deposits of glycogen in the renal tubules. The thyroid appeared normal. One adrenal revealed a single small area of cortical atrophy.

The markedly vacuolated hepatic cells were laden with glycogen (C in figure) and contained practically no fat. The Kupffer cells were fatty. The pancreatic islets were small and not numerous, with the cells showing small dark nuclei and poorly defined scant cytoplasm.

#### COMMENT

The hepatomegaly in the 2 cases reported was associated with deposition of glycogen and may have been related to the insulin hypoglycemia.

Wohlwill<sup>5</sup> reported the presence of large amounts of glycogen in the liver of a diabetic patient who died in insulin hypoglycemia. Duperie and Maupetit<sup>6</sup> described an insulin-treated boy of 7 with glycosuria and acidosis who died in coma and convulsions (hypoglycemic?) and showed a 1,030 Gm liver rich in glycogen and containing only small amounts of fat.

Glycogenic hepatomegaly in a diabetic patient without hypoglycemia has been reported by Brian, Schechter and Parsons<sup>7</sup> as well as by Terplan, Vogel and Hyde,<sup>2a</sup> although the latter entitled their report "Prolonged Diabetes Mellitus Culminating in von Gierke's Disease in a Youth of Childlike Habitus."

Popper and Wozasek<sup>8</sup> reported several cases of diabetes in which the liver contained large amounts of glycogen. Kaufmann<sup>9</sup> stated that occasionally diabetic persons showed large collections of hepatic glycogen and that their livers were large and heavy. Warren<sup>1a</sup> described a 3,900 Gm liver with a normal fatty acid content in a diabetic patient who died of nephritis and pneumonia (case of Dr. F. D. W. Lukens).

5 Wohlwill, F. *Klin. Wchnschr.* **7**: 344, 1928.

6 Duperie, R., and Maupetit. *Bull. et mem. Soc. med. et chir. de Bordeaux* **38**: 317, 1935.

7 Brian, E. W., Schechter, A. J., and Parsons, E. L. *Arch. Int. Med.* **59**: 685, 1937.

8 Popper, H., and Wozasek, O. *Virchows Arch. f. path. Anat.* **279**: 819, 1931.

9 Kaufmann, E. *Spezielle pathologische Anatomie*, Berlin, W. de Gruyter & Co. 1931.

Such observations are not confined to autopsy material, Stetson and Ohler<sup>10</sup> having described a clinical case

Warren<sup>1a</sup> made the following statement

While it has been generally assumed that the increase in size and weight of the liver in diabetes is due to fat, this does not exclude other factors. Enlargement of the liver has been shown to be due to glycogen storage, to circulatory changes, amyloidosis, metastatic tumor as well as fat. Of much importance is the fluid content of the liver. large amounts of fat in the liver do not preclude the presence of normal or even large amounts of glycogen

All of the physiologic processes involved in the deposition of glycogen are not entirely clear, but in the diabetic organism, in the presence of adequate dextrose glycogen is stored in the liver under the influence of insulin. This may have been the mechanism involved in the reported cases

The pancreas was small or the islet tissue scanty in the 2 cases reported here and in several of the other cases cited,<sup>11</sup> as well as in the 2 cases reported by Moore<sup>12</sup> and Bowen and Beck<sup>13</sup>. In the latter

*Pancreatic Weights in Adults with Diabetes*

	Weight of Pancreas					
	To 50 Gm	50-70 Gm	70-90 Gm	90-110 Gm	110-130 Gm	130 Gm Up
Patients with diabetes	9	13	9	14	2	7
Nondiabetic patients	0	6	22	12	8	6

instances both patients were young diabetic subjects with convulsions (hypoglycemic?). In Brian, Schechter and Parsons' <sup>7</sup> case, however, the pancreas was normal

This finding in the group of cases under discussion may have no special significance, for Warren<sup>1a</sup> found the pancreas weighing less than 50 Gm in 74 of a series of 449 cases of diabetes (approximately 16 per cent). In a much smaller series of cases, studied in the department of pathology of the University of Chicago, in which the patients were adults, the pancreatic weights, contrasted with those of a control group, were as shown in the accompanying table

It is interesting that the percentage of cases with the pancreas weighing less than 50 Gm is 16 per cent, duplicating Warren's figure

#### SUMMARY

Two cases in which glycogenic hepatomegaly was associated with insulin hypoglycemia in patients with diabetes are reported, and similar cases in the literature are reviewed

10 Stetson, R. P., and Ohler, W. R. *New England J. Med.* **217** 627, 1937

11 Warren<sup>1a</sup> Terplan and others<sup>2a</sup> Duperie and Maupetit<sup>6</sup> Popper and Wozasek<sup>8</sup>

12 Moore, R. A. *Am. J. Dis. Child.* **52** 627, 1936

13 Bowen, B. D., and Beck, G. *Ann. Int. Med.* **6** 1412, 1933

## LIPOID HISTIOCYTOSIS

### Report of a Case with Postmortem and Chemical Studies of the Spleen

ARCHIE H BAGGENSTOSS, M D , EDWARD F ROSENBERG, M D , AND  
ARNOLD E OSTFEBERG, PH D , ROCHESTER, MINN

In recent years reports of a somewhat ill defined group of diseases characterized by a systemic proliferation of the reticuloendothelial system have appeared in the medical literature with increasing frequency. In most of the cases it has been impossible to establish the cause, and, owing to the varied clinical and pathologic pictures, it has been difficult to classify these diseases satisfactorily. Such attempts have been made, however, by Epstein,<sup>1</sup> Jaffé<sup>2</sup> and others. Sacks, in 1938, basing his classification on Epstein's work, listed the following four groups under the generic term "histiocytomatoses", (1) diseases in which there is disturbance in the storage of lipid, such as Gaucher's disease, Niemann-Pick disease and the Hand-Schuller-Christian syndrome, (2) infectious proliferative granuloma, (3) hyperplasia (reticuloendotheliosis, aleukemic reticulosis), and (4) dysplastic conditions, including so-called endothelioma. Perhaps the best defined and delimited group is the first. The diseases in this category have generally been considered as disturbances in lipid metabolism or storage. In this group, the rather clearcut clinical and histologic pictures have been augmented by exact chemical analysis of the lipoids involved. Thus, Gaucher's disease has been designated as cerebroside lipoidosis, Niemann-Pick disease has been called phosphatide lipoidosis, and the Hand-Schuller-Christian syndrome and xanthomatosis have been termed cholesterol lipoidosis.

That the reticuloendothelial system is chiefly involved in these diseases is admitted by all, as the lipoids are in each instance accumulated within the cells of this system. Whether the disease rests on a primary dysfunction or hyperfunction of the cells of this system or whether the role of the reticuloendothelial system is passive or secondary to some other disturbance, such as one of lipid metabolism, is in dispute and will be considered later. The relationship of lipid histiocytosis (Sacks' group 1) to hyperplasia of the reticuloendothelial system (Sacks' group 3) is also not clear. It is possible that this relationship is more intimate than is generally suspected. Thus, it should be pointed out that in most of the reported cases of hyperplasia of the reticuloendothelial system (Sacks' group 3) the presence of large mononuclear cells in the organs affected is an almost constant observation. Explanations as to why the cells are large or as to what they contain are seldom given. Some authors have stated that the tissues involved are negative to stains for

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From the Sections on Pathologic Anatomy and Clinical Biochemistry of the Mayo Clinic

1 Epstein, E. Med Klin **21** 1501, 1925

2 Jaffe, R. H. The Reticulo-Endothelial System, in Downey, H. Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol 2, p 973

various types of fat. As a rule, however, chemical studies have not been made, and the question as to whether or not these large cells contain lipoids cannot be settled by staining methods. If chemical studies of tissues were carried out in all cases of suspected reticuloendotheliosis, it might help a great deal in clearing up the relationship of these diseases and lead to clearer conceptions as to their genesis.

#### REPORT OF CASE

The patient was a young woman, aged 20 years, who was of English descent. When she registered at the Mayo Clinic, Oct. 23, 1938, because of vomiting which had been present for the past seven weeks, she was admitted directly to the hospital. No relevant information could be elicited regarding the family. Nine siblings were well. Her own past history was likewise irrelevant; she had had pneumonia at the age of 10 years.

Seven weeks before she came to the clinic she began to have attacks of vomiting. At times the attacks were preceded or accompanied by sensations of vertigo. Vomiting increased in intensity. Between Sept. 15 and 23, 1938, she vomited many times daily and also had some fever. No cause of this illness could be determined by her family physician, but, at the insistence of her parents, an exploratory laparotomy was performed on September 23. No lesions which might explain the vomiting were encountered; the appendix, which was the site of slight chronic inflammation, was removed. After the operation the vomiting persisted; it occurred whenever the patient ate, and it frequently was precipitated by quick movements. The patient had a daily fever which continued until she came to the clinic. At times her temperature reached 102° F. For two weeks before the patient came to the clinic slight epistaxis occurred occasionally. On the day of her arrival she noted that her urine was bloody.

When the patient was examined, she was very pale and obviously acutely ill. Her lips were dry and fissured, and numerous petechial hemorrhages were noted about the soft palate, finger tips and left conjunctival sac. The temperature was 102° F. on admission. The pulse rate varied from 100 to 148 per minute during her stay in the hospital. The breasts were not abnormal. The heart was enlarged to the left. There was evidence of congestion at the bases of both lungs. These findings were corroborated by roentgenologic examination of the thorax. There was an enlarged spleen. There was no evidence of ascites, and the pelvis and rectum revealed nothing of importance. The systolic blood pressure was 110 mm. of mercury, and the diastolic pressure was 70 mm.

The specific gravity of the urine was 1.014. There was albuminuria of grade 1, and 12 pus cells appeared in each field of the microscope as viewed under the high power objective. The value for hemoglobin was 6.3 Gm. in 100 cc. of blood. There were 2,090,000 erythrocytes and 11,500 leukocytes per cubic millimeter of blood. The sedimentation rate of the erythrocytes was 120 mm. in one hour. A blood smear disclosed toxic changes, grade 2 to 3, in the polymorphonuclear neutrophils, a shift to the left and immature forms of leukocytes extending as far back as the so-called stem cells. The anemia appeared to be of a secondary type. The appearance of the smear, including the toxic changes in the polymorphonuclear neutrophils and the presence of immature leukocytes, was interpreted as probable evidence of a leukemoid reaction, but the possibility that the patient was suffering from chronic myelogenous leukemia could not be definitely excluded.

The patient failed rapidly. The temperature remained elevated continuously and varied between 100.4 and 103.4° F. until her death, which occurred October 25, two days after her registration.



The body was promptly embalmed, and necropsy was made three hours after death. The body was 163 cm in length and was estimated to weigh 130 pounds (59 Kg). There was no evidence of edema, and the incision in the lower right quadrant of the abdomen had healed. Numerous petechial hemorrhages were seen in the skin over the anterior portion of the thorax, arms and legs, and a somewhat larger effusion of blood was found in the subcutaneous tissues in the midline of the abdomen. The omentum was adherent to the abdominal incision and to the cecum at the site of the recent appendectomy. There was no excess of fluid in the abdominal cavity. Approximately 50 cc of clear straw-colored fluid was found in each thoracic cavity. The lungs were not adherent. Spread irregularly about the pleural surfaces of both lungs were numerous small patches of thickening, which were whitish in appearance and measured 2 to 3 mm in diameter. These had the appearance of patches of leukemic infiltration. The lungs showed slight edema of the lower lobes, but otherwise they were essentially normal in appearance. No evidence of tuberculosis, healed or active, was encountered. The pericardial sac contained approximately 100 cc of clear yellow fluid. The heart weighed 292 Gm. Several minute petechial hemorrhages were noted beneath the pericardium about the anterior surface of the left ventricle. Yellowish subendocardial streaking in the wall of the right ventricle gave the impression of lipid degeneration of the myocardium. Otherwise the heart was not abnormal.

The spleen weighed 704 Gm. Its surface was grayish white and was speckled with white nodules of increased density. These varied in size, some were as large as 4 mm in diameter. The capsule itself was smooth and glistening but otherwise was not thickened. The small nodules rose slightly above the surface of the capsule. Multiple similar whitish nodules were distributed widely about the cut surface and had replaced a considerable quantity of the splenic pulp (fig 1). The remaining splenic substance was light red.

The liver weighed 2,570 Gm. Its surface was studded irregularly with occasional nodules, which were as large as 3 or 4 mm in diameter and were similar in appearance to those noted in the spleen. The gross appearance of the sectioned surface was not abnormal.

The marrow in the vertebrae of the thoracic and lumbar segments of the spinal column contained numerous grayish yellow irregular softened regions which varied from a few millimeters to 3 cm in diameter.

The lymph nodes about the hili of the lungs and about the pancreas, mesentery and aorta were not enlarged. The entire gastrointestinal tract and the pancreas, kidneys, adrenal glands, ureters, bladder, uterus, thymus and thyroid gland were normal. There were multiple follicular cysts in both ovaries. Atherosclerosis of the aorta, grade 1, was present. The brain weighed 1,147 Gm and appeared normal throughout. The spinal cord was normal in appearance. The pituitary body likewise appeared normal.

*Histologic Observations*—The most significant histologic changes were in the spleen, bone marrow, liver, lungs and lymph nodes.

(a) Spleen. Paraffin sections which were stained with hematoxylin and eosin revealed almost complete destruction of the normal architecture by a massive proliferation of large, faintly staining cells, which appeared as a syncytium in many regions (fig 2A). These cells were present apparently throughout the spleen, and in the grossly visible grayish white nodules they were the only elements to be seen. In regions in which the cellular hyperplasia was less advanced the proliferating cells appeared to take their origin from the cytoplasmic reticulum rather than from the cells lining the sinuses. Occasional large reticular cells were seen lying in the sinuses, however.

The abundant cytoplasm of the hyperplastic cells was faintly eosinophilic or neutrophilic and contained numerous tiny vacuoles which failed to take the stain (fig 2B). The boundaries of these cells were not distinct, and in many regions in which the cells were closely packed no definite borders could be seen. In some cells the cytoplasm extended out into long strands or sail-like projections, while other cells, which were more closely packed, were polyhedral or oval. In general, the cells were large and varied from 10 to 18.5 microns in diameter.

The nuclei, although rather large, appeared small in relation to the cytoplasm. They generally measured about 7 microns in diameter. Most of them were oval or kidney shaped. The chromatin was arranged as a network, there were condensations of it at various points which gave the nucleus a granular appearance. The Pappenheim and Dominici<sup>2a</sup> methods of staining revealed the delicate nature



Fig 1—At the top is seen a cross section of the spleen, showing nodules and hypertrophy, at the bottom, a cross section of a normal spleen

of the chromatin network and emphasized the vacuolation of the cytoplasm. Multinucleated cells were numerous, and some of them appeared similar to the Sternberg-Reed cells of Hodgkin's disease (fig 2C). Other characteristic features of Hodgkin's disease, such as eosinophils, fibrosis and necrosis, were absent. The homogeneity of the cellular elements tended to eliminate the presence of Hodgkin's disease.

Some of the proliferated cells appeared to have phagocytic ability, as they contained erythrocytes and cellular debris. In sections which were stained for iron many of the cells were found to contain hemosiderin in varying amounts. In the regions in which proliferation appeared most rapid there was very slight or no evidence of phagocytosis.

Sections impregnated with silver by the methods of de Galantha<sup>3</sup> and Bielschowsky revealed a network of reticulum fibers in intimate relationship with these cells. In regions in which proliferation of the cells led to the formation of distinct nodules these fibers were few in number and very fine. Sections stained by Mallory's phosphotungstic acid hematoxylin stain likewise revealed these reticulum fibers.

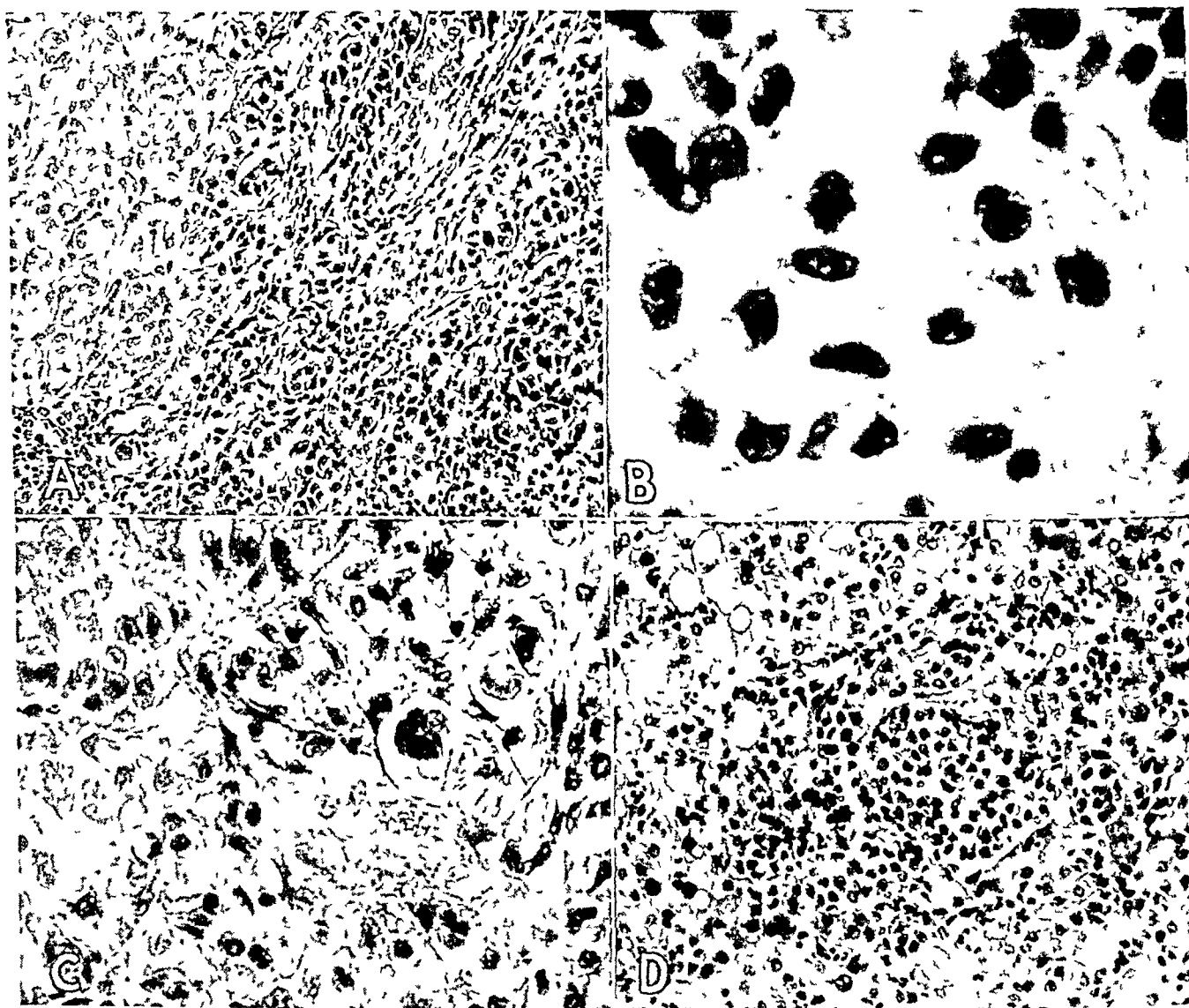


Fig 2—*A*, section obtained from the edge of a nodule in the spleen, hematoxylin and eosin,  $\times 180$ . *B*, same section as shown in *A*,  $\times 1,000$ . *C*, giant cells in the spleen, hematoxylin and eosin,  $\times 450$ . *D*, proliferation of cells in the liver, stained with hematoxylin and eosin,  $\times 200$ .

Frozen sections stained with sudan III and scarlet red did not reveal any fat. The results obtained with the Lorrain Smith-Dietrich stain were inconclusive as to the presence of lipoids, but sections stained by Ciaccio's method revealed small

<sup>3</sup> de Galantha, E. Personal communication to the authors.

yellowish orange droplets in the cytoplasm of some of the cells. These are supposedly indicative of the presence of phosphatides. The results obtained with Weigert's myelin sheath stain were negative.

(b) Liver. Sections of this organ were interesting, as the proliferating cells, although identical in appearance and staining reactions with those of the spleen, appeared to arise from the Kupffer cells lining the sinusoids (fig 2 D). In sections taken from the edge of the nodules which were grossly visible there were definite transitions between the Kupffer cells and the hyperplastic elements. Some of these cells were still attached to the walls of the sinusoids, while others were lying free in the lumen. In some regions the proliferation was so advanced that the sinusoids were distended by solid groups of cells. In other regions in which the cells retained their connection with the walls of the sinuses the hyperplasia had resulted in the formation of pseudoacini.

(c) Bone marrow. Sections were obtained from the sternum, vertebrae and the petrous portion of the temporal bone. The cells were similar in appearance and staining reactions to those in the spleen and liver (fig 3 A). The staining methods of Pappenheim and Dominici demonstrated vacuolated cytoplasm and a faintly staining delicate network of chromatin in the nucleus. The cells appeared to arise from the cells lining the sinusoids and formed solid groups and pseudoacini like those seen in the liver. Multinucleated cells and mitotic figures were numerous. In some regions the cellular hyperplasia had completely replaced the normal marrow, and there was resorption of the trabeculae. There was no evidence of anything resembling normal hemopoiesis in these proliferating elements. In one of the vertebrae the process had broken through the cortical bone and had lifted up the periosteum.

Touch preparations of sternal and vertebral marrow were also made and stained with Wright's stain and Giemsa's stain. These preparations contained numerous large cells with faintly staining vacuolated cytoplasm and nuclei which had a very delicate, finely stippled chromatin network. The majority of the cells had the typical structure of reticuloendothelial cells.

(d) Lymph nodes. The only lymph nodes which appeared to share in the hyperplasia were those at the hilus of the lung. In these the proliferation had resulted in the formation of nodules, which were not sharply demarcated but seemed to fuse gradually with the surrounding lymphatic tissue. In the region of this nodular hyperplasia the normal architecture of the node had completely disappeared. Some of the nodes showed profuse proliferation and mobilization of the littoral cells lining the lymph sinuses, similar to that seen in various inflammatory processes (fig 3 B). In some regions the newly proliferated cells, both the reticulum and the lining cells, contained large amounts of carbon pigment (fig 3 C). The staining reactions of the cells were identical with those of the cells of the spleen.

(e) Lungs. Sections through the macroscopically visible nodules in the lungs revealed regions of cellular hyperplasia identical with those already described (fig 3 D). In addition, there were small foci of cellular proliferation about the small blood vessels. In these foci the cells appeared to arise from the adventitia of the vessels.

In many regions the alveolar walls appeared thickened as a result of an increased number of large mononuclear cells, which possessed vacuolated cytoplasm and appeared similar to those already described. Considerable numbers of these cells were also free in the alveoli and contained phagocytosed erythrocytes and hemosiderin. In other portions of the lung there was evidence of edema, and in a few

foci there was a slight fibrinous exudate, which contained very few cellular elements, chiefly mononuclear cells

(f) Breast and ovary In routine sections of the left breast and right ovary there were a few minute foci of cellular proliferation which were identical with those already described These cells appeared to arise from the connective tissue

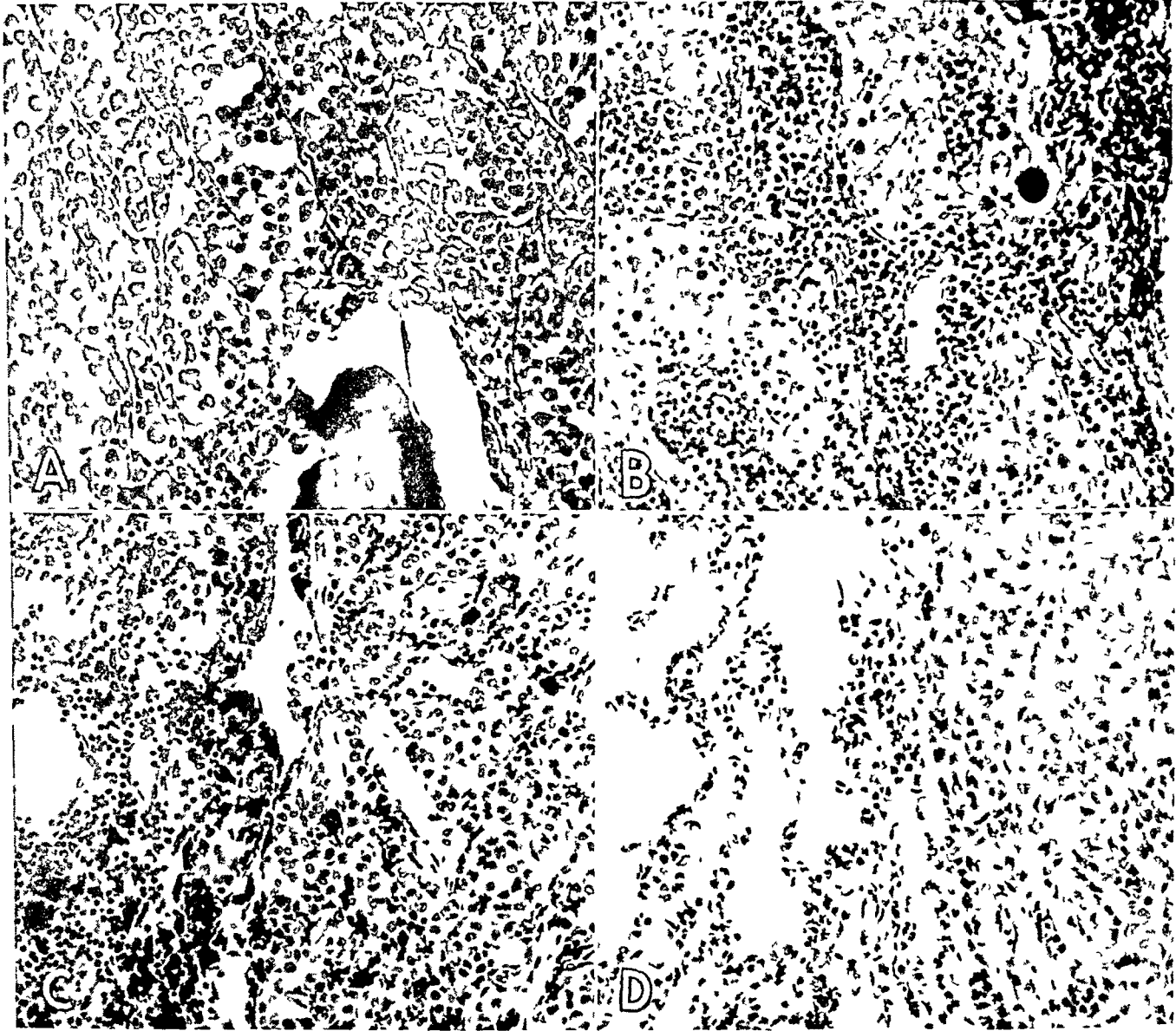


Fig 3—*A*, proliferation of cells in sinuses of the bone marrow, hematoxylin and eosin,  $\times 200$  *B* proliferation and mobilization of littoral cells in sinuses of a hilar lymph node, hematoxylin and eosin,  $\times 200$  *C*, proliferating cells in a lymph node, some of which reveal phagocytosis of carbon pigment, hematoxylin and eosin,  $\times 200$  *D*, section of a pulmonary nodule, hematoxylin and eosin,  $\times 200$

(g) Heart Frozen sections of the right ventricle of the heart stained with sudan IV revealed numerous fine lipid granules in the muscle fibers

(h) Other organs Sections of the kidneys, adrenal glands, thymus, uterus, pancreas, thyroid gland and bladder were normal

In view of the fact that the histologic appearance of the organs was in many respects similar to that of the tissues in Gaucher's disease, the spleen was analyzed for lipoids according to the method of Lieb<sup>4</sup>. At the same time an analysis for lipoids was made on a spleen which had been removed at operation in a case of Gaucher's disease. In table 1 (prepared from the data of Epstein and Lorenz<sup>5</sup>) are presented chemical data which distinguish the lipid diseases. In this table it may be seen that Niemann-Pick disease is associated with a marked increase in the phosphatide and lecithin whereas Gaucher's disease is distinguished by a very large content of cerebroside kersin. In the Hand-Schuller-Christian syndrome, which is one of the xanthomatoses, cholesterol and cholesterol esters are stored in the diseased tissues

TABLE 1—*Concentration of Kersin and Lipoids in a Normal Spleen, in a Spleen Involved in Niemann-Pick Disease, in a Spleen Involved in Gaucher's Disease and in a Mass Removed from Dura Mater Involved in the Hand-Schuller-Christian Syndrome*

	Concentration, Expressed as Percentage of Weight of Dried Organ or Tissue			
	Normal Spleen	Spleen Involved in Niemann Pick Disease	Spleen Involved in Gaucher's Disease	Mass from Dura Mater Involved in Hand Christian Schuller Syndrome
		Phosphatide Lipoidosis	Cerebroside Lipoidosis	Cholesterol Lipoidosis
Kersin	0	0	10	0
Cholesterol	0.62	0.73	Trace	3.2
Cholesterol esters	0.287	0.68	Trace	15.3
Total cholesterol	0.9	1.41	Trace	18.58
Lecithin	1.66	13.17	1.46	1.60
Neutral fat	4.183	12.82	Trace	14.4
Total lipoids *	4.6	12.91	Trace	31.58

\* The figures for this were obtained as follows: 10 per cent was subtracted from the value for neutral fat. This 10 per cent represents the approximate weight of glycerol in the fat molecule. To the resulting figure was added the value for total cholesterol. Thus for the spleen of the patient with Niemann-Pick disease observed by Epstein and Lorenz the calculations were as follows: spleen 12.82 - 1.3 = 11.5 (total fatty acids) 11.5 + 1.41 = 12.91 (total lipoids). The values for total lipoids obtained in this manner may be compared with the values for total lipoids in table 2, and indicate the total combined weight of fatty acids and cholesterol with cholesterol esters.

in large quantities. In table 2 are presented the results of an analysis of the two spleens. Both show an increase in total lipoids and cholesterol (table 1). The spleen in the case of Gaucher's disease contained a large quantity of the cerebroside kersin whereas only a small amount of this was found in the spleen in the present case. Lecithin<sup>6</sup> was found in significant amounts in the spleen in the present case but not in the spleen in the case of Gaucher's disease.

4 Lieb, H. Ztschr f physiol Chem **140** 305, 1924

5 Epstein, E., and Lorenz, K. Ztschr f physiol Chem **192** 145, 1930

6 Klenk (Baumann, T. Klenk, E., and Scheidegger, S. Ergebn d allg Path u path Anat **30** 183, 1936) recently claimed that the phospholipid which is found in the cells in Niemann-Pick disease is not composed entirely of lecithin but consists largely of the diamminophosphatide sphingomyelin. Baumann (Baumann, T. Klin Wchnschr **14** 1743, 1935) and Tropp (Tropp, C. ibid **15** 562, 1936) have confirmed this. In order to simplify our study, the entire ether-soluble phospholipid fraction of the spleen is reported as lecithin.

## COMMENT

Consideration of the data in tables 1 and 2 suggests that the disease which we have reported could not be classified definitely in any of the groups of disorders of the storage of lipoids. The presence of considerable quantities of lecithin and of a large amount of total lipoids suggests Niemann-Pick disease, but this is probably excluded by the clinical data, for Niemann-Pick disease has been noted for its tendency to affect infants and to cause death during the first few years of life. From a chemical standpoint, the dysfunction of fat metabolism in our patient was probably of a type intermediate between that of Gaucher's disease and that of Niemann-Pick disease.

In recent years a number of workers have indicated that from a chemical standpoint an overlapping of these three diseases occurs frequently. Thus Sobotka, Epstein and Lichtenstein<sup>7</sup> pointed out that in many of the reported cases of Niemann-Pick disease an examination of tissues has disclosed not only an accumulation of lecithin but also a considerable increase in the amount of cholesterol (3.1 to 6.6 times the normal amount). Clément<sup>8</sup> agreed with them in this opinion and said that as observations multiply one sees that the original classifications do not take care of all cases, as delimitations are artificial and many clinical phenomena overlap. Hamperl<sup>9</sup> also reported an instance in which overlapping of the metabolic fault was definitely present. In a case of Gaucher's disease he found a considerable amount of lecithin in the spleen (5.98 per cent), nearly as much as Bloom and Kern<sup>10</sup> found in a case of Niemann-Pick disease. Pick<sup>11</sup> also held this view, in the Dunham lecture, which was delivered at Harvard University in 1932, he stated that a number of deviations which exist do not fit into rigid schemes. Our data suggest that the disease condition reported belongs in this group of deviations mentioned by Pick.

This condition presented a difficult diagnostic problem to both the clinician and the pathologist. The symptoms and signs suggested an infection, and subacute bacterial endocarditis was considered, but this diagnosis could not be substantiated. Acute leukemia was suggested, but this diagnosis was not definitely supported by the blood smears, which pointed rather to a leukemoid reaction or to chronic myelogenous leukemia. The rapid downhill course precluded further laboratory studies, and the patient died before a definite diagnosis could be made.

At necropsy the disease likewise presented difficulties in diagnosis. No infectious process could be demonstrated. The enlarged spleen and the nodules in the liver, lungs and bone marrow, considered together with the findings on the blood during life, suggested chronic myelogenous leukemia. Frozen sections of these tissues, however, did not support this diagnosis but pointed rather to lipid histiocytosis, possibly Gaucher's disease. For this reason the chemical studies were carried out, with results as already noted (table 2).

7 Sobotka, H., Epstein, E. Z., and Lichtenstein, L. *Arch Path* **10** 677, 1930.

8 Clément, R. *Rev de med, Paris* **55** 219, 1938.

9 Hamperl, H. *Virchows Arch f path Anat* **271** 147, 1929.

10 Bloom, W., and Kern, R. *Arch Int Med* **39** 456, 1927.

11 Pick, L. *Am J M Sc* **185** 601, 1933.

Further histologic study did not support the initial impression that we were dealing with a typical form of either Gaucher's disease or Niemann-Pick disease. The lipid containing cells were not as large as those described for these diseases, while the nuclei were larger than those usually described. The vacuolated cytoplasm of some of the cells, however, resembled that found in Niemann-Pick disease. Ciaccio's method of staining revealed phospholipoid in occasional cells. This also suggested Niemann-Pick disease. None of the cells possessed the fibrillated cytoplasm characteristic of Gaucher's disease. The presence of numerous mitotic figures indicated more active hyperplasia than is usually seen in lipid histiocytosis. The microscopic appearance of the lesions was similar in many respects to that of a neoplastic process and the possibility of retothelial sarcomatosis as outlined by Roulet<sup>12</sup> and Benecke<sup>13</sup> was considered.

In the bone marrow, especially, the behavior of the hyperplastic process, including the resorption of bone trabeculae and actual erosion

TABLE 2—Concentration of Kerasin and Lipoids in a Spleen Involved in Gaucher's Disease and in a Spleen Involved in Atypical Lipoid Histiocytosis

	Concentration, Expressed as Percentage of Weight of Dried Organ	
	Spleen Involved in Gaucher's Disease	Spleen Involved in Atypical Lipoid Histiocytosis <sup>*</sup>
Kerasin	11.0	0.37
Cholesterol	2.42	1.97
Lecithin	Trace	2.20
Total fatty acids	6.14	9.30
Total lipoids	8.57	11.10

\* Unfortunately the spleen had already been fixed in modified Kaiserling's solution and consequently the values obtained for lecithin were probably lower than the actual amounts present at the time of death. The content of kerasin, cholesterol and neutral fat in tissues is not altered by exposure to fixing solutions.

of the cortex, was that of a neoplasm. The lesions in the liver and spleen, however, resembled hyperplasia of preexisting Kupffer cells and reticulum cells rather than a new growth. Multiple myeloma was also considered, but the diagnosis could not be substantiated. Clinically and histologically there were many similarities between this case and the cases of acute reticuloendotheliosis reported by Uehlinger,<sup>14</sup> Ugriumow,<sup>15</sup> Sacks<sup>16</sup> and others. Jaffé summarized the available information from such cases, and it is not necessary to review the data here. Suffice it to say that the insidious onset, weakness, fever, petechiae, splenomegaly, inconclusive hematologic picture and hyperplasia of the cells of the reticuloendothelial system in this case are all features which have been described in many reports of cases of acute reticuloendotheliosis which he

12 Roulet, F. Virchows Arch f path Anat **286** 702, 1932

13 Benecke, E. Virchows Arch f path Anat **286** 693, 1932

14 Uehlinger, E. Beitr z path Anat u z allg Path **83** 719, 1930

15 Ugriumow, B. Zentralbl f allg Path u path Anat **42** 103, 1928

16 Sacks, M. S. Arch Path **26** 676, 1938



has reviewed. If determinations of the concentration of lipid in the spleen had not been carried out in this case, the disease would probably have been considered as acute reticuloendotheliosis.

Some of the clinical symptoms of the patient can be explained by the observations at necropsy. The cause of the persistent vomiting is not certain. Because of the involvement of the marrow of the petrous portion of the temporal bone it was thought that there might be interference with the vestibular apparatus of the internal ear. This deduction could not be substantiated, however, although numerous sections were taken through the internal ear. The anemia, petechiae and epistaxis were probably the result of the reduction of functioning marrow by the hyperplastic process.

The point that should be emphasized here is that this condition clinically and histologically fell under the heading of hyperplasia of the reticuloendothelial system as classified by Sacks<sup>16</sup> (group c) but that when chemical determinations were made the unusual quantities of lipid constituents of the spleen placed the disease in the group of lipid histiocytosis. To us, this suggested the possibility that if lipoids were determined in all cases of reticuloendotheliosis a great many more disease conditions would be placed in this group rather than in that of simple hyperplasia. It also suggested the possibility that the relationship between these two groups may be much more intimate and fundamental than was believed.

The widely accepted view of Epstein and Pick<sup>17</sup> is that the diseases known as lipid histiocytosis (Gaucher's disease, Niemann-Pick disease, and the Hand-Schüller-Christian syndrome) are disturbances of the storage of lipid. Thus Pick advanced the theory that the role played by the reticular cells is of secondary importance and that the primary condition is a disturbance of metabolism. In other words, the substance which appears in the cells is not the product of abnormal activity of these cells but arises extracellularly and is merely stored by these cells. Recent investigation suggests, however, that this view may be in error inasmuch as there is now some evidence of abnormal metabolism in these cells. It is known, for instance, that the cerebroside kerosin is a galactoside derived from the ceramid lignoceryl-sphingosin by combination with galactose. Fraenkel and Bielschowsky<sup>18</sup> demonstrated lignoceryl-sphingosin in the normal liver, and, according to Thannhauser,<sup>19</sup> this substance occurs only in organs that are rich in reticular cells. Tropp<sup>20</sup> said that since lignoceryl-sphingosin is a normal product of the reticuloendothelial cells, it may be that in these cells the fermentative synthesis of the galactoside kerosin takes place. Tropp assumes that normally the kerosin which is formed by the reticuloendothelial cells is carried to the nerve tissues to be used for the building up of myelin. In Gaucher's disease, then, the kerosin accumulates in the organs of the reticuloendothelial system either because of a disturbance in the mobilization and transportation of the finished product to the brain, spinal cord and

17 Pick, L. *Ergebn d inn Med u Kinderh* **29** 519, 1926.

18 Fraenkel, E., and Bielschowsky, F., cited by Baumann, Klenk and Scheidegger in footnote 6.

19 Thannhauser, S. J. *Klin Wchnschr* **11** 1693, 1932.

20 Tropp, C. *Klin Wchnschr* **15** 562, 1936.

myelinated nerves or because of excessive formation of kerafin by the reticuloendothelial cells. Likewise in Niemann-Pick disease, Dienst and Hamperl<sup>21</sup> and Tiopp assume that the dysfunction of the reticuloendothelial system is primary and the lipid changes are secondary.

Recent work indicates also a close basic relationship between Gaucher's disease and Niemann-Pick disease. Klenk,<sup>20</sup> for example, was able to show that the organs in Niemann-Pick disease contained large amounts of the diaminophosphatide sphingomyelin. In the liver it formed 23.8 per cent and in the spleen 24.7 per cent of the dried substance, whereas the glycerophosphatides (such as lecithin) were not increased. Baumann and Tiopp<sup>20</sup> reported similar findings. It was pointed out by these men that sphingomyelin and kerafin have as a common base lignoceroyl-sphingosin. In Gaucher's disease this base combines with galactose to form kerafin, while in Niemann-Pick disease it combines with choline to form the diaminophosphatide sphingomyelin.

According to this hypothesis, the amount and character of the lipoids in the affected organs depend on the synthetic activity of the reticuloendothelial cells. Depending on the direction and degree of this activity, different types and amounts of lipoids might be produced. Such a hypothesis would explain conditions such as that encountered in our case which fits neither group of diseases satisfactorily.

There appears to be a need for thorough chemical studies of the involved tissues not only in cases of lipid histiocytosis but in all cases of reticuloendotheliosis, whether or not disturbances in lipid metabolism are suspected. We feel that such studies may lead to more complete information regarding the nature of reticuloendotheliosis and its relation to lipid metabolism.

#### SUMMARY

The atypical lipid histiocytosis which we have reported does not fit into the present classification of such diseases. Other reports which have appeared indicate that in these diseases there is considerable overlapping as regards the character and amount of the lipid constituents of the organs involved. Recent investigation also tends to show that dysfunction or unexplained hyperplasia of the reticuloendothelial system may be primary and play an active rather than a passive part in the genesis of these diseases. It is believed that if chemical determinations were carried out in all cases of so-called primary hyperplasia of the reticuloendothelial system as well as in cases of typical lipid histiocytosis a better understanding of these disorders might result.

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21 Dienst, G., and Hamperl, H., cited by Jaffe.<sup>2</sup>

# Laboratory Methods and Technical Notes

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## A COMPARISON OF STARCH PASTE AND ALBUMIN MIXTURE AS AGENTS FOR THE ROUTINE MOUNTING OF PARAFFIN SECTIONS

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The albumin-glycerin mixture<sup>1</sup> for affixing paraffin sections to slides has been used routinely in many pathologic laboratories and has undoubtedly been one of the best preparations for this purpose. However, since Spoerri<sup>2</sup> suggested the use of a starch paste for mounting sections of nerve tissue, the use of such a starch suspension has been tried in this laboratory for routine affixation of paraffin sections of all surgical pathologic specimens. It is because of the advantages afforded by the use of this suspension that we feel a comparison of the methods is warranted.

The albumin-glycerin mixture<sup>1</sup> is made up by taking equal parts of egg white and glycerin, filtering, and adding a small crystal of thymol as a preservative. The starch paste tried is essentially the same as the one described by Spoerri. One gram of powdered starch is added to 10 cc of cold water and thoroughly mixed, the solution is then poured into 20 cc of boiling water, 2 drops of dilute hydrochloric acid is added, and the suspension is boiled for five minutes while being constantly stirred to free the opalescent suspension from lumps of starch. A small crystal of thymol should be added after the paste has cooled.

The clean glass slides are prepared by coating one surface with a thin film of the mixture to be used. Drying of the affixative on the slides does not seem to be a disadvantage as long as no dust is allowed to collect on them.

Paraffin sections are then flattened out on the surface of warm water and floated onto the slides already smeared with the adhesive to be used. Sections thus mounted on slides are ready for drying prior to their deparaffinization and staining.

Several methods of drying have been tried, but it is felt that the following are the most effective and time saving in the particular staining technic concerned, although it is recognized that longer drying periods at lower temperatures may be used successfully. For a modified Masson trichrome stain,<sup>3</sup> the ordinary hematoxylin and eosin method, a modified Giemsa procedure<sup>4</sup> and the Foot silver impregnation for brain tissue,<sup>5</sup> the following drying method is considered effective and adequate.

Rest one end of each slide on the edge of a tray so that the slides will be in a slanting position. The tray with the slides is then put on an electric hot plate

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1 Meyer, P. Mitt zool Station, Neapel **4** 521, 1883

2 Spoerri, R. Science **90** 260, 1939

3 Goldner, J. Am J Path **14** 237, 1938

4 Wolbach, S. B. J M Research **41** 1, 1919-1920

5 Foot, N. C. Am J Path **14** 245, 1938

and left there for about three minutes after the paraffin has begun to melt. The sections attached to the slides in such a manner are then put into an oven and kept at 56 to 58 C for one hour or longer. The sections are now ready for deparaffinization before staining.

A comparison of the adhesive qualities of the albumin and the starch affixatives shows that there is no advantage of one over the other in the staining procedures noted.

With the Ziehl-Neelsen carbol fuchsin method,<sup>6</sup> the three minutes on the hot plate and one hour in the drying oven at 56 to 58 C are found to be effective and adequate when the albumin preparation is used, but longer drying is necessary when starch is used as an adhesive. After the sections are floated onto the slides filmed with the starch paste, the slides are put in an oven and kept at 38 to 40 C for about twenty-four hours. The sections are then found to adhere well while being subjected to this staining technic.

*Table Illustrating Temperature and Time Necessary for Drying Sections to Insure Good Adhesion of Paraffin Sections to Slides During Use of Staining Technics*

Drying Method	Affixative	Modified Masson Stain <sup>1</sup>	Hematoxylin Eosin	Modified Giemsa <sup>4</sup>	Foot Silver for Brain <sup>5</sup>	Ziehl Neelsen <sup>6</sup>	Foot Silver for Reticulum <sup>7</sup>
On hot plate for 3 min and in drying oven at 56-58 C for 1 hr	Albumin	A	A	A	A	A	I
	Starch	A	A	A	A	I	I
In drying oven at 38-40 C for 24 hr	Albumin	A	A	A	A	A	I
	Starch	A	A	A	A	A	I
In drying oven at 56-58 C for 48 hr or at 38-40 C for 5 days	Albumin	U	U	U	U	U	A
	Starch	U	U	U	U	U	A

A = adequate, I = inadequate U = unnecessary

With the Foot method of silver impregnation for reticulum,<sup>7</sup> it is found that the albumin and the starch paste are equally effective as far as their adhesive qualities are concerned. However, a longer drying time has been found necessary for either affixative used. After sections have been floated onto the prepared slides and incubated at 56 to 58 C for forty-eight hours, they adhere well. This is important as the chief complaint of technicians using this impregnation has always been directed against the detachment of sections from the glass slides.

The starch paste has no such affinity for any of the dyes in the various staining technics used as is occasionally seen with the albumin mixture, and therefore gives a definitely clearer background. This is especially true when the sections are impregnated with silver, the egg albumin tends to combine with the silver while the starch does not.

The staining properties of the various tissues are the same whether starch or albumin affixatives are used, but the most notable feature resulting in the starch-filmed slides is the clear background.

<sup>6</sup> Mallory, F. B. *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938, p. 276.

<sup>7</sup> Foot, N. C., and Menard, M. C. *Arch. Path.* 4: 211, 1927.

The fixation of tissues in formaldehyde-alcohol solution, Zenker's fluid or Bouin's fluid makes no variation in the adhesiveness with either paste used

Other advantages of the starch over the albumin are its easy preparation, simplicity and cleanliness, which render it most suitable for routine affixing of paraffin sections to slides

#### SUMMARY

It has been shown that a starch paste has some advantages over the albumin-glycerin mixture for affixing paraffin sections to slides in the course of several staining techniques

## Notes and News

**University News, Promotions, Resignations, Appointments, Deaths, Etc**—Max Askanazy, professor of pathologic anatomy at the University of Geneva, Switzerland, has reached the age limit and retired. His pupil Erwin Rutishauser was chosen as his successor.

Perrin Long has been appointed head of a new department, that of preventive medicine, in the school of medicine of Johns Hopkins University, Baltimore.

Fritz Strassmann, professor of forensic medicine at the University of Berlin from 1894 to 1930, when he retired, died Jan 30, 1940, in his eighty-second year.

Bruno Galli-Valerio, professor of hygiene and bacteriology at the University of Lausanne, Switzerland, has retired after fifty years of teaching. Paul Hauduroy, director of the health department of Colombes, near Paris, has been appointed as his successor.

Alexander Besredka, of the Pasteur Institute in Paris, died Feb 28, 1940, at the age of 70.

**Medicolegal Conference**—February 23, a medicolegal conference was held in Chicago under the auspices of the Committee on Local Medicolegal Problems of the Institute of Medicine, Oscar T. Schultz, chairman. In the evening Alan R. Moritz, professor of legal medicine, Harvard Medical School, delivered an address on "Medical Science and the Administration of Justice."

**Research in Problems of Sex**—Applications to the Committee for Research in Problems of Sex, of the National Research Council, for financial aid in support of the study of fundamental problems of sex and reproduction during the year beginning July 1 should be received before April 1. They may be addressed to Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Preference will be given to proposals for investigation of neurologic, psychobiologic and behavior problems.

**Leon Bernard Prize**—The first award of the Leon Bernard Prize of the Health Committee of the League of Nations has been made to Wilbur A. Sawyer, director of the International Health Division of the Rockefeller Foundation, for his work on yellow fever and in the general field of preventive medicine.

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Experimental Pathology and Pathologic Physiology

CYTOLOGICAL CHANGES INDUCED IN THE HYPOPHYSIS BY PITUITARY EXTRACT  
A E SEVERINGHAUS and K W THOMPSON, *Am J Path* **15** 391, 1939

The hypophyses studied were from (a) 2 dogs given daily injections of 25 cc of an extract of sheep pituitary for one hundred and twenty and two hundred and ten days, respectively, (b) 2 dogs treated subcutaneously for thirty-two and thirty days, respectively, with 10 cc of "antihormone" serum from a dog that was treated daily for three years with an extract of sheep pituitary, and (c) 2 immature, 4 month old female sheep given daily injections of 25 cc of an extract of sheep pituitary for six months. The cytologic changes in the first pair of animals were noted mainly in the basophilic cells. The granules clumped to aggregates of varying sizes, and vacuoles appeared in the cytoplasm, some colorless, some filled with a clear pale blue substance and some filled with a deeply basophilic amorphous material. The vacuoles tended to expand and to occupy most of the granular cytoplasm. In some cells these changes were identical with those observed in the hypophysis of the castrated rat or monkey, in other cells they resembled closely the changes seen in the pituitary gland of the thyroidectomized rat, still other cells showed the hyaline-like cytoplasmic changes that were described by Crooke in the Cushing syndrome of pituitary basophilism. The hypophyses of the second pair of animals presented a decrease in the number of chromophobe cells and tinctorial and structural changes in the basophilic and in the acidophilic cells, both types of cells occurring in increased numbers. Another change was marked hyperemia and edema. In the hypophyses of the 2 immature female sheep the most notable feature was an almost universal degranulation of the basophilic cells. Changes were also noted in the size and staining qualities of the acidophilic cells. A hypothesis is offered to explain the observed changes. The injected pituitary extract activates the endocrine glands related to the anterior lobe of the pituitary, namely, the gonads, the thyroid and probably the adrenals, which activation stimulates in turn the anterior lobe of the hypophysis to increased secretion. The injected heterologous pituitary extract stimulates the production of "antihormones," which neutralize at first the injected extract, they lose later their species specificity and neutralize the pituitary secretion of the treated animal itself. The changes in the second pair of animals, accordingly, would be due to the injected "antihormones." The result is a state analogous to pituitary insufficiency, with atrophy of the gonads, thyroids and adrenals. The latter leads to cytologic changes in the anterior lobe of the pituitary as described.

I DAVIDSON

IDIOPATHIC FAMILIAL LIPEMIA L E HOLT JR, F X AYLWARD and H G  
TIMBRES, *Bull Johns Hopkins Hosp* **64** 279, 1939

A girl of 11 years was found to have extreme lipemia—due primarily to neutral fat—associated with hepatomegaly, splenomegaly, psoriasis, peculiar ulcerations of the skin and malnutrition. She suffered from periodic acute attacks of abdominal pain, which were accompanied by a sudden reduction in the blood fat, enlargement of the liver and spleen and engorgement of the abdominal veins. There was evidence that the disorder was familial. The condition was not improved by administering lecithin, choline, thyroxin, insulin, "lipocatic" or an

extract of the anterior lobe of the pituitary. It could, however, be in large measure controlled by a low fat diet. Some evidence was obtained of a beneficial effect from blood transfusion. The authors discuss the various types of disturbance which may give rise to lipemia. They suggest that the condition described is due to a defect in the mechanism of the removal of blood fat by the liver—a mechanism in which a humoral factor appears to be concerned.

FROM AUTHORS' SUMMARY

RADIOACTIVE IRON AND ITS METABOLISM IN ANEMIA. P. F. HAHN, W. F. BALE, E. O. LAWRENCE and G. H. WHIPPLE, *J. Exper. Med.* **69** 739, 1939.

Artificially produced radioactive iron is an extremely sensitive agent for use in studying the course of iron through its changes in body metabolism, the radioactive iron lending itself to observations of absorption, transport, exchange, mobilization and excretion. The need of the body for iron determines in some manner the absorption of this element. In a normal dog in which there is no need for the element it is absorbed in negligible amounts. In an anemic animal it is promptly assimilated. The plasma is clearly the means of transport of iron from the gastrointestinal tract to the point of mobilization of the element for fabrication into hemoglobin. The speed of absorption and of transfer of iron to the red cell is spectacular. The importance of the liver and of the bone marrow in the metabolism of iron is confirmed.

FROM AUTHORS' SUMMARY

EXTRAVASCULAR DEVELOPMENT OF THE MONOCYTE OBSERVED IN VIVO. R. H. EBERT and H. W. FLOREY, *Brit. J. Exper. Path.* **20** 342, 1939.

Ebert and Florey, using a modified Sandison-Clark cell, have watched the extravascular development of individual monocytes into cells indistinguishable from tissue histiocytes. They produce evidence to show that macrophages appearing in organizing tissue do not originate from the histiocytes previously present in surrounding normal tissue.

INTESTINAL REACTIONS TO GASTRIC JUICE. H. W. FLOREY and others, *J. Path. & Bact.* **49** 105, 1939.

Various technical procedures are described for comparing the resistance of the duodenum to gastric juice with that of the jejunum and ileum, and it is shown that the duodenum has a much greater resistance than other parts of the intestine. These findings are consistent with the view that Brunner's glands are concerned in a mechanism protective against gastric juice. Histologic and endoscopic observations on parts of the intestine exposed to gastric juice are described.

FROM AUTHORS' SUMMARY

TRANSPLANTATION OF THE TESTICULAR AND THE OVARIAN FATTY BODY. F. X. HAUSBERGER, *Virchows Arch. f. path. Anat.* **302** 640, 1938.

In the rat at birth there is present beside the head of the epididymis or beside the ovary a small collection of cellular tissue which later becomes the testicular or the ovarian fatty body. At birth it consists of a syncytium of fibrocytes and histiocytes with few blood vessels. On the fifth to sixth day the tissue becomes more highly vascularized, and proliferation leads to the formation of localized cellular areas, in which fat begins to be deposited on the seventh day. The fat is deposited in the form of minute droplets in cells of the histiocyte type, but only in certain of these. In the adult the fatty body is a characteristically lobulated, pedunculated mass of adipose tissue, which is larger in the male than in the female. To study the development of adipose tissue, the fatty bodies of rats 2 to 3 days old were transplanted into the peritoneal cavities of rats weighing 50 to 80 Gm. Only the pedicle of the minute fatty body was sutured to the peritoneum. The transplanted fatty bodies grew and developed exactly as in the normal animals.



Fat was deposited in the cells, the bodies took on the characteristic lobulation and reached a size equal to that of the normal fatty body. The fully developed transplanted male fatty body reached a size larger than that of the female body, no matter whether it was transplanted into a male or a female rat. Transplanted connective tissue of young rats was not transformed into adipose tissue. The author concludes that adipose tissue develops from cells specifically differentiated for that function, probably through the development of specific intracellular enzymes.

O T SCHULTZ

## Pathologic Anatomy

THE LYMPHOCYTE IN ACUTE INFLAMMATION IN THE RABBIT F KOLOUCH JR,  
Am J Path **15** 413, 1939

A transformation of clasmatocytes to histogenous macrophages is the initial response of the rabbit in acute inflammation. The majority of the macrophages in the exudate associated with the acute inflammatory process are of hematogenous origin. The lymphocyte-macrophage transformation occurs early in the course of the inflammation. By the fourteenth hour the lymphocytic origin of many mononuclear cells in an inflamed area is largely obscured. In studies made eighteen hours or later after the onset of an acute inflammation in a tissue, cell lineage cannot be traced. The employment of tissue spreads, dried and stained like blood smears, allows a comparison of the cells in an acutely inflamed tissue with cells of blood smears.

FROM AUTHOR'S CONCLUSIONS

THE PITUITARY IN MONGOLISM C E BENDA, Arch Neurol & Psychiat **42** 1,  
1939

Benda considers mongolism a pituitary disorder resulting from deficiency of the basophilic and chief cells of the anterior lobe of the hypophysis. The cells are reduced in number, with the result that there is an apparent increase of the eosinophilic cells. Benda arrived at this conclusion from a study of 14 patients, in 13 of whom mongolism was unquestionably present. The histologic changes were identical regardless of the age (from a few days to 30 years) and were the reverse of what is seen in Cushing's disease, in which the number of the basophilic cells is increased. The condition of the thyroid has no bearing on the histologic changes in mongolism.

GEORGE B HASSIN

FATAL HYPOGLYCEMIA A L SAHS and L ALEXANDER, Arch Neurol & Psychiat  
**42** 285, 1939

In a fatal case of hypoglycemic shock of twenty-eight hours' duration, the benzidine staining method revealed a number of anomalies in the blood vessels and capillaries—hyperemia, irregular dilatation, perivascular hemorrhages, variations in the appearance of the capillaries and blood vessels, thrombi and so-called "white stasis," i. e., early thrombosis in which white blood cells are enmeshed in scanty threads of fibrin. Ganglion cells showed ischemic degeneration, and in the cortex of the central and upper parietal regions there were foci of blanching. The nerve fibers were well preserved, but the axons were "pushed apart as though by interstitial edema." The authors consider the blanching, the edema and the changes in the ganglion cells secondary to the vascular changes and due to local and general anoxia.

GEORGE B HASSIN

TEETH IN OSTEOGENESIS IMPERFECTA M A RUSHTON, J Path & Bact **48** 591,  
1939

Teeth from a woman who had suffered from late osteogenesis imperfecta are described. They were small, with pinkish crowns and short translucent roots. The odontoblasts had differentiated normally, though the growth of the whole dentinal

papilla was reduced. Normal matrix and Tomes' fibrils were produced at the periphery of the teeth, but soon the odontoblasts and other cells concerned ceased to form Tomes' fibrils and normal matrix. This change occurred first where the odontoblasts were oldest, but in a piecemeal manner. Focal defects in the rate of formation of the matrix led to the inclusion therein of blood vessels. The peripheral pulp cells produced precollagenous argyrophil fibers, but these were not converted into collagen except in the immediate proximity of blood vessels. Pulp stones of good collagen content occurred in the middle of the dentinal papilla. The matrix was inadequately calcified and probably lacking in cementing substances. At a later stage, when no normal Tomes' fibrils were being formed, a tissue resembling primitive fiber bone replaced the former pulp cavity. Most of the dentinal tubules which had been formed became occluded.

FROM AUTHOR'S SUMMARY

EFFECT OF ACUTE AND CHRONIC URINARY RETENTION ON THE KIDNEY. K. HELMKE,  
Virchows Arch f path Anat **302** 323, 1938

As the result of a histologic study, Helmke reaches the conclusion that chronic stasis of urine has an effect on the kidney quite different from that of acute retention. The latter causes single or multiple ruptures of the calices in the region of the fornix and leads to a reflux of urine directly into the veins of the pelvis. The escape of urine into the blood stream may lead to acute collapse. The path of escape may be detected roentgenologically. If infection does not occur, the process heals when the cause of the acute retention is relieved. In urinary retention of more gradual onset and longer duration the retained urine distends the glomerular capsules and escapes into the interstitial tissue of the kidney. From here it makes its way into the lymphatics and finally into the veins. In the veins and lymphatics the material has the same optical and staining properties as in the tubules and glomerular capsules, Helmke terms these masses venous and lymphatic cylinders or casts. The constituent common to tubular casts and venous cylinders is urinary mucoid. The veins and lymphatics of the interstitial tissue play an important part in the spread of ascending infections of the kidney.

O. T. SCHULTZ

HISTOLOGY OF CHRONIC PEPTIC GASTRIC AND DUODENAL ULCER. A. THELEN,  
Virchows Arch f path Anat **302** 515, 1938

A histologic study of gastric and duodenal ulcer was made in 32 resection specimens by a variety of staining methods. Especial attention was paid to the formation and composition of the characteristic zone in the floor of the ulcer to which Askanazy had given the name "layer of fibrinoid necrosis." In recent years Klinge and many others have applied the term "fibrinoid" to a change in connective tissue, occurring usually in hyperergic inflammation, characterized by transformation of connective tissue into fibrillated argentophil material. In this sense Askanazy's layer of fibrinoid necrosis is not the result of fibrinoid degeneration. The layer is formed by swelling of the connective tissue without the formation of fibrinoid, the swollen fibers undergoing necrobiosis. The author terms this process *Quellungsnecrose* (swelling necrosis). On the surface of the layer of necrosis there may be deposited a layer of exudate composed of true fibrin, leukocytes and erythrocytes, beneath the zone of necrosis is the zone of proliferative inflammatory reaction. The layer of necrosis may be demarcated and sequestered, a new necrotic layer being formed. The acid gastric secretion is a factor in the formation of the necrotic layer, but apparently not necessarily directly, since an ulcer may reveal necrosis in one portion and healing in another. Thelen postulates a disturbance of the correlation gastric juice-gastric wall.

O. T. SCHULTZ

## Microbiology and Parasitology

TUBERCLE BACILLI IN NON-TUBERCULOUS LUNG TISSUE W H FIDMAN and A H BAGGENSTOSS, *Am J Path* **15** 501, 1939

The results of this study, which was made of material from an area where the morbidity from tuberculosis is not high, indicate that virulent tubercle bacilli are infrequently present in the presumably nontuberculous tissue of the lungs of persons dying of causes other than tuberculosis

FROM AUTHORS' SUMMARY

INTRACELLULAR BACILLI IN TYPHOID FEVER J W ADAMS JR, *Am J Path* **15** 561, 1939

Gram-negative bacillary forms, judged to be *Eberthella typhi*, have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon and mesenteric lymph nodes in 5 cases of early typhoid fever. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early classic intestinal and mesenteric lesions of typhoid fever.

FROM AUTHOR'S SUMMARY

EFFECT OF ULTRAVIOLET RADIATION ON TUBERCLE BACILLI K C SMITHBURN and G I LAVIN, *Am Rev Tuberc* **39** 782, 1939

The effect of approximately monochromatic ultraviolet radiation (2,537 angstroms) on human tubercle bacilli in saline suspension was studied. Heavy suspensions of tubercle bacilli (1 mg per cubic centimeter) required relatively long periods of irradiation (ten minutes or more) before the organisms were rendered nonviable, weaker suspensions, a shorter time. Organisms killed by ultraviolet radiation retained the property of acid-fastness. Bacilli in heavy suspensions were rendered avirulent only after relatively long exposure to ultraviolet radiation, but those in weak suspensions were quickly reduced in virulence. Reduction in virulence could be demonstrated after less irradiation than is required to kill the organisms, and the organisms could be avirulent without being killed. Irradiated viable organisms possessed the capacity of inducing demonstrable immunity. Organisms killed by the radiation did not induce measurable immunity.

H J CORPER

VITAMIN C AND IMMUNITY IN TUBERCULOSIS OF GUINEA PIGS F H HEISE and W STEENKEN JR, *Am Rev Tuberc* **39** 794, 1939

Vitamin C given subcutaneously and in abundance does not influence the course of tuberculosis in guinea pigs infected with 10,000 H 37 Rv bacilli, does not influence the content of vitamin C in the blood serum and does not influence the sensitivity to tuberculin. Rotter's test proved of no value in differentiating super-vitaminosis C.

H J CORPER

PRESERVATION OF TUBERCLE BACILLI M L COHN, *Am Rev Tuberc* **40** 99, 1939

Desiccated human (virulent and avirulent), bovine (virulent) and avian (virulent) tubercle bacilli at refrigerator temperature retain their viability almost completely for three years, while natural cultures at the same temperature survive only about six months to one year (occasionally two years). At incubator temperature the loss of viability of the desiccated cultures of these same strains of tubercle bacilli is much more rapid, being almost complete at six months to one year, while at room temperature such cultures survive a little longer than at incubator temperature. The loss of viability of tubercle bacilli is primarily a function of the temperature at which they are stored regardless of whether they are desiccated or natural. The rate of the loss of viability of cultures is greater in the presence of oxygen, less in the presence of air and least in the presence of nitrogen. Desiccation, although a minor factor, aids in maintaining the viability. Desiccation can

remove as much as 70 to 75 per cent of the water (and volatile materials) from the cultures without appreciable detrimental effect on the viability. Aside from mammalian bacilli, cultures of avian tubercle bacilli can be preserved in viable form for over three years when desiccated and sealed in air or nitrogen and maintained at refrigerator temperature. The method possesses the advantage that the strains occupy little space and are readily transportable. H. J. CORPLER

THE SPREAD OF TUBERCLE BACILLI IN SENSITIZED AND IMMUNIZED ANIMALS  
J. FREUND and D. M. ANGINI, *J. Immunol.* **35** 271, 1938

Rabbits were given several intracutaneous injections of heat-killed tubercle bacilli and were tested for hypersensitiveness one week after the last injection of old tuberculin. They were then inoculated intradermally with living tubercle bacilli, bovine or human, of varying degrees of virulence. Normal rabbits were similarly inoculated with living tubercle bacilli at selected intervals. Immunized and control rabbits were killed, and the skin at the site of injection and the inguinal nodes were removed and examined for tubercle bacilli culturally and histologically. In the previously immunized animals the tubercle bacilli multiplied and were more abundant in the skin after the same interval than in the infected animals that were previously normal. In the immunized animals the bacilli reached the regional lymph nodes from two days to two weeks later than in the normal controls. It appears that this retardation was caused by local fixation of the bacilli and not by destruction. I. DAVIDSOHN

PURIFICATION OF INSECT-TRANSMITTED PLANT VIRUSES  
F. C. BAWDEN and N. W. PIRIE, *Brit. J. Exper. Path.* **20** 322, 1939

From plants infected with potato virus Y, which is an insect-transmitted virus, Bawden and Pirie have isolated a liquid crystalline protein with many properties similar to those of potato virus X, although it is much less stable. The isolation is complicated by the small amount of virus in infective sap and by the presence of other constituents having properties in common with the virus.

ACTION OF BILE SALTS ON VIRUSES  
W. SMITH, *J. Path. & Bact.* **48** 557, 1939

Certain bile salts are able to inactivate some viruses but have no apparent effect on others. The inactivation is almost instantaneous and is thought to depend on lysis of the virus elements. Sodium deoxycholate and sodium apocholate are the most active of the bile salts investigated, the former being about four times as active as the latter. Sodium cholate possesses only slight activity. The susceptibility of a virus to bile salts is not related to its size. The process of lysis by a bile salt can be observed both macroscopically and microscopically with the cultivable virus-like organism of pleuropneumonia and the sewage organisms of Lairdlaw and Elford. Attempts to use preparations of lysed virus for prophylactic immunization have so far not been encouraging. FROM AUTHOR'S SUMMARY

TUBERCULOUS SUPERINFECTIONS  
V. REYNES, *Ann. Inst. Pasteur* **62** 177, 1939

Reynes studied the reactions which appear in superinfected guinea pigs from the eleventh to the eighty-sixth day after the first inoculation with fragments of autogenous bacillary lesions (autosuperinfections) or fragments of tuberculous lesions developing on other animals (heterosuperinfections). Contrary to descriptions by other authors, he shows that the lesions and reactions were the same in these guinea pigs whether the animals were tested with their own organisms or with those of other animals. Superinfections occurring in the three weeks following the primary inoculation develop like primary infections and may lead to the production of a local lesion of superinfection if the quantity of bacilli is sufficient. Superinfections occurring a month after primary inoculation are usually without effect. Test organisms are then either destroyed or blocked in the tissues in which they are deposited or in which the culture may be found after a longer

or shorter period. Sometimes, also, the immunity created by a primary infection is indicated only by an obstacle to the dissemination of the inoculated organisms, there is then a longer lapse of time before the ganglions in the superinfected animal are attacked than in the control animal. Finally, this immunity may be forced when the material inoculated contains a large number of bacilli, i. e., the organisms not only multiply where inoculated but reach the neighboring lymph nodes and produce lesions.

FROM AUTHOR'S SUMMARY

### Immunology

PRODUCTION OF KIDNEY ANTIBODIES. F. F. SCHWINTER and F. C. COMPTON, *J. Exper. Med.* **70** 223, 1939

Rabbits given injections of emulsions of homologous kidney to which staphylococcus or streptococcus toxins had been added produced complement-fixing antibodies which reacted with both rabbit kidney and brain. By absorption tests it was demonstrated that the serum contained at least two antibodies, one specific for kidney and the other nonspecific. Similar antibodies for kidney were found in the blood of a majority of patients with scarlet fever but in the blood of only a few normal persons. The possibility that a similar or related antibody may be etiologically concerned in scarlatinal nephritis is discussed.

FROM AUTHORS' SUMMARY

SPECIFIC ANTISERUM FOR BLOOD-GROUP FACTORS A, B, M AND N. W. C. BOYD, *J. Immunol.* **37** 65, 1939

Satisfactory anti-A agglutinating serum was produced relatively easily in rabbits, i. e., in somewhat less than 50 per cent of the animals, by inoculating them with human A red cells. The serum was absorbed to remove the anti-B, anti-M and anti-N agglutinins. Anti-B serum was also produced but not quite as easily. In an attempt to produce anti-A and anti-B heteroagglutinins by injecting other antigens than human red cells into animals, rabbits and roosters were inoculated with human or horse saliva containing blood group factors A or B, with peptone (Witte) containing factor A and with rabbit red cells known to contain a fraction of the human factor B. In response to human saliva A and to peptone, highly group-specific agglutinins were produced. The anti-B serum was less satisfactory. Methods of preservation of serum, including the anti-M and anti-N testing fluids, are offered which made it possible to keep them in usable strength for as long as five and one-half years.

I. DAVIDSOHN

IMMUNOLOGICAL RELATIONSHIP OF THE VIRUS OF SPONTANEOUS COWPOX TO VACCINIA VIRUS. A. W. DOWNIE, *Brit. J. Exper. Path.* **20** 158, 1939

Downie's finding that histologic differences occurred in the lesions produced by the viruses of cowpox and vaccinia led him to investigate these two viruses by serologic methods. He now shows that rabbits immunized with either virus are immune to both and that immune serum prepared with either virus neutralizes both. However, cross absorption experiments with hyperimmune serum and suspensions of elementary bodies show that while the homologous virus removes the antibodies for both viruses, the heterologous virus absorbs chiefly its own antibodies. These results indicate that the viruses of cowpox and vaccinia are closely related but not identical as previously assumed by many workers.

ACTION OF PROTEOLYTIC ENZYMES ON THE ANTITOXINS AND PROTEINS IN IMMUNE SERUM. C. G. POPE, *Brit. J. Exper. Path.* **20** 201, 1939

In the second paper dealing with the action of proteolytic enzymes on antitoxic serum Pope describes the results obtained after a limited amount of enzyme action on antitoxins followed by critical heat denaturation of nonantitoxic protein.

Several enzymes possess the property of altering the antitoxic pseudoglobulin and converting it into two protein fractions, one of which is nonantitoxic and easily denatured in acid solutions at high temperatures. The antitoxin is not destroyed by this treatment.

COMPLEMENT IN NEPHRITIS C E KILLETT and J G THOMSON, *J Path & Bact* 48 519, 1939

In 38 cases of nephritis the complementary activity of the serum for sensitized sheep cells was estimated. The cases were subdivided into groups on clinical grounds and the classification confirmed when possible by autopsy. In every case of acute glomerulonephritis examined within four weeks after the onset complementary activity was found to be much lower than normal.

FROM AUTHORS' SUMMARY

## Tumors

INFLUENCE OF DINITROKRESOL ON THE DEVELOPMENT OF TAR TUMORS IN MICE L KREYBERG, *Am J Cancer* 36 51, 1939

Sixty mice of a genetically known strain have been painted with tar and treated with large doses of dinitrocresol (from 0.1 to 2 mg per day). The tumor response corresponds closely to that of similar animals treated with dried thyroid gland, namely, a general tendency to earlier formation of tar tumors.

FROM AUTHOR'S SUMMARY

EFFECT OF HYPOPHYSIAL TRANSPLANTS IN VARIOUS STRAINS OF MICE L LOEB and M M KIRTZ, *Am J Cancer* 36 56, 1939

The processes of normal and cancerous growth which take place in bearers of anterior hypophysial transplants are in certain respects more similar to those taking place spontaneously in mice than to those induced by injections of estrogen. In both the so-called spontaneous tumors and those induced by transplanting anterior lobe of the hypophysis the hormones which represent the effective stimuli originate in the animals themselves, whereas in tumors induced by injecting estrogen the source of effective stimulation is extrinsic.

FROM AUTHORS' SUMMARY

MORTALITY FROM CANCER OF THE SKIN K K CONRAD and A B HILL, *Am J Cancer* 36 83, 1939

A comparison is made between the mortality from cancer of the skin and lip and that from cancer of other sites in different occupational groups. The reported inverse association between these forms of cancer is not confirmed. On the contrary, although numerous exceptions occur, there is on the average a slight direct association, occupations in which there is a relatively high incidence of cancer of the skin and lip tending to show also an excess of cancer of other sites.

FROM AUTHORS' SUMMARY

ASPIRATION BIOPSY OF TUMOR OF THE LIVER J S BINKLEY, *Am J Cancer* 36 193, 1939

Aspiration biopsy for tumor of the liver has been done in 19 cases. Sufficient material was obtained by aspiration to establish a diagnosis in 73.6 per cent of the cases. Follow-up records of the clinical course in each case support the histologic diagnosis. Aspiration biopsy of hepatic tumors has not been associated with significant complications in the hands of 10 different clinicians who have used the method on one or more occasions.

FROM AUTHOR'S SUMMARY

STUDIES IN CARCINOGENESIS HYDROCARBON-CHOLESTEROL PELLETS IN ALBINO MICE M J SHEAR and EGON LORENZ, *Am J Cancer* **36** 201, 1939

Cholesterol pellets containing 5 per cent dibenzanthracene produced tumors in the course of a year in a considerable proportion of the mice in which they were implanted. Similar pellets containing 1 per cent dibenzanthracene produced tumors in only a small percentage of mice. The two 1 per cent pellets which produced tumors contained 0.12 mg and 0.48 mg of dibenzanthracene, respectively. Spectrographic analysis of these pellets, recovered after the induction of tumors, showed that there was still some dibenzanthracene present. No tumors were obtained with cholesterol pellets containing 0.1 and 0.01 per cent, respectively, of dibenzanthracene. The significance of the one sarcoma obtained with a 0.001 per cent dibenzanthracene-cholesterol pellet is therefore obscure.

FROM AUTHORS' SUMMARY

STUDIES IN CARCINOGENESIS COMPOUNDS RELATED TO 3,4-BENZOPYRENE M J SHEAR, *Am J Cancer* **36** 211, 1939

Twelve derivatives of 3,4-benzopyrene were tested for carcinogenic potency by injecting them subcutaneously into pure strain mice. Of these derivatives, the 4'-methyl and the 1',2'-dihydro-4'-methyl derivatives were found to be carcinogenic. The results obtained with 80 other polycyclic compounds are briefly recorded. The findings are discussed from the point of view of the relationship of these synthetic compounds to substances of biologic origin and from that of the implications as regards the mechanism of tumor genesis.

FROM AUTHOR'S SUMMARY

EXPERIMENTAL ZINC TERATOMA OF THE TESTIS L I FRAIN and K E GROMZEWA, *Am J Cancer* **36** 233, 1939

The injection of small amounts of 10 per cent zinc sulfate solution (0.15 to 0.2 cc) into the genital glands of fowl produces in a considerable percentage (15 per cent of all birds treated in March) rapidly growing tumors of these glands. Being composed of a great variety of tissues—epithelial and glandular elements, cartilage, developing bones, smooth muscle tissue, pigment cells, embryonic connective tissue, anlagen of nerve elements—these tumors must be classified as teratoid neoplasms and in this respect are quite similar to the teratoma produced by Michalowsky and others with zinc chloride. The production of teratoid tumors of the testes by injections of either zinc chloride or zinc sulfate solution shows that the ions  $\text{Cl}_2$  and  $\text{SO}_4$  do not play any particular role in the production of tumors by zinc salts in the genital glands of fowl. Further experiments must show whether other substances having a similar influence on the tissues but having nothing in common with the zinc salts in their chemical structure may produce a similar effect. (See also the article on experimental zinc teratoma of the testis by V. Anissimova [*Am J Cancer* **36** 229, 1939].)

FROM AUTHORS' SUMMARY

ANGIORETICULOENDOTHELIOMA (KAPOSI'S DISEASE) OF THE HEART R M CHOISSE and E M RAMSEY, *Am J Path* **15** 155, 1939

The problem of the origin and of the nature of the lesions of Kaposi's disease has been reanalyzed on the basis of a review of the literature on the subject and in the light of a thorough study of 2 recent cases, in both of which the disease was primary in the right auricle of the heart and in which lesions of the skin were lacking. It is concluded that the condition is a true neoplasm, derived from the reticuloendothelial system, with neoformation of blood vessels a prominent distinguishing characteristic. Since tumors are properly named with respect to their tissues of origin, it is proposed that in the future the scientific term "angioreticuloendothelioma" be used in preference to the term "Kaposi's disease."

FROM AUTHORS' SUMMARY

CARCINOMA OF THE PANCREAS R D'AUNOY, M A OGDEN and B HALPERT,  
*Am J Path* 15 217, 1939

In 6,050 autopsies on persons over 1 year of age 40 cases of primary carcinoma of the pancreas were encountered. Males and females were represented in the series in the proportion of 7:1. Twenty-three of the patients were over 60 years of age. The average duration of illness was four and a half months. Thirty-one neoplasms were situated in the head and 9 in the tail. All were columnar cell carcinoma. Carcinoma primary in the head of the pancreas readily invaded the duodenum, and that primary in the tail spread over the peritoneum. Metastases were observed in the liver in 25 instances.

FROM AUTHORS' SUMMARY

FOLLICULAR LYMPHOBLASTOMA AND A RELATED FORM OF LYMPHOSARCOMA S  
MAYER JR and H M THOMAS JR, *Bull Johns Hopkins Hosp* 64 315, 1939

Five cases of follicular lymphoblastoma are reported. In this series follicular lymphoblastoma has not proved to be entirely benign, despite the usual dramatic early response to radiotherapy. The length of response to irradiation cannot be prophesied from the presence or absence of microscopic evidence of invasiveness. The possibility of clinical recognition of follicular lymphoblastoma is emphasized. The authors describe 4 cases of malignant lymphoblastoma of an unusual but characteristic type. The patients presented the following features: general or local lymphadenomegaly, splenomegaly due to the presence of tumor cells, which were localized especially in the malpighian bodies, lymph nodes showing diffuse infiltration with lymphoblastic cells, a normal blood picture except for moderate anemia, a rapidly fatal course.

FROM AUTHORS' SUMMARY

INTESTINAL POLYPS GENESIS AND RELATION TO MALIGNANT GROWTHS R J  
COFFEY and J A BARGEN, *Surg, Gynec & Obst* 69 136, 1939

A study of a group of cases of multiple adenomatosis and of polyposis associated with chronic ulcerative colitis revealed a striking dissimilarity both clinically and pathologically. Of the cases of multiple adenomatosis, a heredofamilial disposition was present in 34.5 per cent. It is essentially a disease of youth, approximately two thirds of the patients being in the first three decades of life. Instances of the condition developing in later life were not uncommon, and the growths were indistinguishable from those in adolescents. The individual lesion consisted of a primary epithelial change with minimal evidence of inflammation. Hypertrophic lymph follicles seemed to have a part in the genesis of the polyps. Characteristic of the condition are myriads of true adenomatous polyps. Carcinomatous transformation was demonstrable in 62.5 per cent, in 25 per cent multiple carcinomas occurred. Histologically it appeared that multiple small foci of adenomatous proliferation develop in a usually hyperplastic mucosa. For this reason the condition is a disease of the entire mucosa, and eradication of only the existent polyps fails to cure the process.

Polyposis complicating chronic ulcerative colitis is characterized by widespread inflammation and destruction of the mucosa with inflammation of the whole wall. The polyps consist of tufts of granulation tissue and of surviving remnants of mucosa, commonly showing benign regenerative hyperplasia, often with adenomatous or even carcinomatous transformation. In this group 56.2 per cent were classed as pseudoadenomatous, 21.9 as true adenoma and 21.9 per cent as carcinomatous. Polyps associated with chronic ulcerative colitis seem to be the result of widespread ulceration and destruction of the mucosa, associated with remaining islets of inflammatory mucous membrane, followed by cicatricial distortion of the lining. It seems that multiple adenomatosis is potentially a much more dangerous condition with respect to carcinoma than polyposis associated with chronic ulcerative colitis.

FROM AUTHORS' SUMMARY (WARREN C HUNTER)



TWO RARE TUMORS OF THE THYROID UMEDA, Virchows Arch f path Anat  
302 458, 1938

The tumors described are a papillary carcinoma, which occurred in the thyroid of a 7 year old boy, and a fibro-osteochondrosarcoma, which occurred in a woman aged 68. Skeletal metastasis did not occur in either case, and evidences of thyroid deficiency were not observed.

O T SCHULTZ

DAMAGE OF LUNGS FOLLOWING ROENTGEN IRRADIATION H VOEGT, Virchows  
Arch f path Anat 302 468, 1938

In a patient with mammary carcinoma and another with Hodgkin's disease, both of whom had been subjected to therapeutic roentgen irradiation of the chest, marked fibrosis of the lungs in the areas previously irradiated was found at autopsy. This is considered the end stage of damage done to the lungs at the time of irradiation.

O T SCHULTZ

EXTRAMEDULLARY PLASMOCYTOMA H VOEGT, Virchows Arch f path Anat  
302 497, 1938

Five examples of extramedullary plasmocytoma and 1 example of plasmocytic granuloma are described. Of the 5 neoplasms, 3 arose in the upper air passages, 1 in the maxillary antrum and 1 in the thyroid (the first recorded instance of such a tumor in this organ). The true tumors are composed of cells of a single type, the plasma cell, which may reveal considerable pleomorphism.

O T SCHULTZ

### Medicolegal Pathology

THE DURET-BERNER HEMORRHAGES B DAHL, Deutsche Ztschr f d ges gerichtl  
Med 29 366, 1938

Berner, in his early observations on hemorrhages occurring in the floor of the fourth ventricle after injuries to the head, believed these hemorrhages impaired the function of the vital medullary centers and directly caused death. In 1936 he questioned the importance of such hemorrhages as lethal agents and suggested that they might be meaningless agonal collections of blood resulting from diapedesis. Duret produced hemorrhages in the floor of the fourth ventricle and in other parts of the brain by injecting quickly into the subdural space a large volume of liquid. The similarity of the hemorrhages observed by Duret and Berner under widely different circumstances led to the employment of the hyphenated name "Duret-Berner" to designate a special type of extravasation. In investigating hemorrhages of this nature, Dahl chose 50 corpses in widely divergent groups as concerned terminal illness and mode of death.

The subjects in the first group died suddenly from coronary sclerosis, pulmonary emboli, convulsions with epilepsy, gunshot wounds, hanging, acute alcoholism and strychnine poisoning. The second group was afflicted with acute illnesses, such as septicemia, otitis media, hemorrhagic diathesis and acute infections and intoxication. The third group died of chronic illnesses, such as carcinoma, sarcoma, tuberculosis, pernicious anemia and chronic cardiac and renal ailments. The results of the studies of the brains in the three groups were strikingly similar. Hemorrhages occurred regularly in the pia, especially in the sulci, where small collections of blood apparently were due to diapedesis and larger collections came from actual tears in the venules, which could be demonstrated in serial sections.

Rows of veins were grossly visible in the floor of the fourth ventricle in front of the stria acoustica. These veins had an extra-wide perivascular space which had to be traversed by tributary venules. In the vicinity of these veins ring

hemorrhages were most common. They drained to a focal point in the pons, and the resulting cluster did not allow all the veins to empty at once. As a result, some veins became overdistended and ruptured. Similar hemorrhages were found in the lung, mesentery and other parts of the body. As a rule, quick death is associated with numerous hemorrhages in the brain, as are ailments which lower the resistance of the veins—e. g., leukemia, hemorrhagic diathesis and anaphylactic shock. Still other conditions cause hemorrhages by an increase in venous pressure as encountered in strangulation, forcible respiration and compression of the abdomen.

Experiments on guinea pigs and rabbits failed to produce hemorrhages in the brain similar to those seen in human beings. An explanation for this is found in the fact that in these animals the veins in the vicinity of the fourth ventricle are distributed evenly and do not cluster in a pontile knot as do the veins in man. Dahl believes that the hemorrhages observed by Berner and Duret are not the same. He contends that the Berner hemorrhages are not the cause of death but are the result of the true cause of death, as are agonal hemorrhages elsewhere.

GEORGE J. RUKSTINAT

CEREBRAL CHANGES IN CASES OF SUDDEN DEATH. A. WELZ, *Virchows Arch f path Anat* **302** 657, 1938

In 4 cases of sudden death no adequate gross cause of death could be discovered at necropsy. The persons had previously been apparently healthy. Microscopic examination revealed a form of encephalitis characterized by perivascular lymphocytic infiltration of the brain and meninges. The condition is not believed to be a specific form of encephalitis. The author's concept is that some previous infection had altered the reactivity of the vessels, causing them to react by a cellular exudative process to some factor, such as trauma, anesthesia, intoxication or infection, insufficient in itself to cause death. The end effect is looked on as a cerebral circulatory death.

O. T. SCHULTZ

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

ALFRED PIAUT, *President*

CHARLES T. OICOTT, *Secretary*

*Regular Meeting, Nov 30, 1939*

### Subacute Endocarditis with Systemic Moniliosis S. H. POLAYES

A white man 48 years of age, a drug addict for twenty years and, in the parlance of drug addicts, a "main line shooter" (inoculating himself intravenously) was brought into the Cumberland Hospital, of Brooklyn, April 24, 1939, complaining of epigastric pain, vomiting and fever of two days' duration. Since January 1939 he had been suffering from pains in joints and muscles and was gradually becoming weaker. He had had gonorrhea twenty-nine years before, diphtheria twelve, a cardiac murmur four and pneumonia two years before. His father died of tuberculosis.

This man presented evidence of chronic illness, a "spiking" temperature, a pulse rate of 114 and 28 respirations per minute. The blood pressure was 90 systolic (there is a question as to the diastolic pressure). There were blue spots in the skin along the veins of the arm. A rough aortic systolic murmur was heard. A roentgenogram of the heart showed a mitral configuration. There was abdominal tenderness to deep palpation. The skin showed crops of petechiae. There were transitory cutaneous nodules in the palms and soles. There were splenic enlargement, sternal tenderness, pains in joints and chills. Death followed seven weeks after admission.

During the course of this man's illness each of thirteen blood cultures showed pure colonies of monilia. The rest of the laboratory data are not pertinent. A biopsy of the skin through the blue specks revealed a foreign body granulomatous reaction caused by iron-free particles of debris (probably introduced with the needle).

On postmortem examination the heart showed the most interesting of all the changes. The right posterior cusp of the aortic valve was almost completely replaced by a cauliflower-like mass of vegetations composed of monilia, leukocytes and fibrin. The mass projected 4 cm upward into the lumen of the aorta, at the base it was continuous with a fluctuating mass of necrotic structure and colonies of monilia, which produced a bulging of the right atrial wall, perforating at a point just above the medial cusp of the tricuspid valve.

The complete list of anatomic diagnoses follows: monilial endocarditis, fibrosis and calcification of the aortic valve, acute interstitial myocarditis and pericarditis, myofibrosis cordis, infarcts of the spleen and kidneys, hemorrhagic pneumonitis, acute hepatitis, meningoencephalitis and hemorrhages of the spinal cord, aneurysm of the superior mesenteric artery, subcutaneous foreign body granulomas of the upper extremities.

#### DISCUSSION

AMOUR F. LIBER: Was any attempt at therapy made?

S. H. POLAYES: We used sulfanilamide, if that is what you mean.

AMOUR F. LIBER: No, there are several other treatments for mycoses.

### Hyperparathyroidism in Infancy and Childhood DOROTHY H. ANDERSEN (by invitation)

Within recent years patients with hyperparathyroidism have been found to be divisible into two groups: (1) those whose condition is due to a primary adenoma of the parathyroid, and (2) those whose condition is due to hyperplasia of the

parathyroid, secondary to prolonged severe renal insufficiency. In the first group the symptoms may be relieved by parathyroidectomy, but in the second group such relief is only temporary. In both groups there are bone changes of a similar nature, a disturbance of the calcium and phosphorus metabolism and sometimes metastatic calcification. The concept that prolonged renal insufficiency produces hyperplasia and hyperactivity of the parathyroids is on a firm basis. Within recent years it has been recognized that renal rickets is a form of renal hyperparathyroidism occurring in late childhood and bears the same relationship to adult renal hyperparathyroidism that gigantism does to acromegaly.

A case is reported of renal hyperparathyroidism in an infant dying at the age of 6 months of calcification of the arteries, including the coronary arteries. This case presents the essential characteristics of the disease: (1) a severe renal insufficiency, due in this instance to congenital hydronephrosis and cystic kidneys, (2) hyperplasia of the parathyroids, (3) high serum phosphorus and nonprotein nitrogen, low serum calcium and low carbon dioxide-combining power, (4) the bone changes of mild hyperparathyroidism, and (5) metastatic calcification of the middle-sized arteries. Death was apparently due to a coronary infarct. The chief points of difference from the adult disease were the lesser degrees of parathyroid hyperplasia and of bone changes.

### Architecture of the Amyloid Kidney    JEAN R. OLIVER

This presentation consisted of a demonstration of drawings and photographs of microdissections of amyloid kidneys.

The places of deposit of the amyloid in the early and late stages of the disease were shown, and it was emphasized that the deposition of the amyloid cannot be an infiltration which proceeds from certain points in the vascular tree but is a scattering of minute deposits which ultimately fuse. The new formation of vascular branches from the arteries direct to the tubular circulation was also demonstrated.

The distortion of nephrons is similar in character to that seen in other forms of chronic Bright's disease. The processes of hypertrophy and hyperplasia are, as in these other forms, limited to the proximal convolution. Aglomerular tubules are also present. These parenchymal distortions seem to be chiefly the effect of a reactive inflammatory process occurring in the interstitial substance of the organ.

An examination of the topography of the amyloid kidney shows that the same sorts of metallaxis are found in it as in other forms of chronic Bright's disease.

### DISCUSSION

PAUL KLEMPERER: I should like to ask whether the preparations came from kidneys which were small. Or were they still large? I ask the question because patients with so-called amyloid contracted kidney rarely have a history of amyloid nephrosis. I wonder whether there are differences in the development of the contraction of the kidney in amyloidosis, therefore I should like to know whether the kidneys were small.

SILIK H. POLAYES: I should like to ask whether it is not possible that some of the infiltrate of the so-called interstitial nephritis which was observed might represent a coincidental infiltration which preceded the deposition of amyloid. I ask that because one finds it so often in the kidney, not related to the pathologic alterations produced in the amyloid kidney.

ALFRED PLAUT: I do not know if Dr. Oliver will like my question, but I cannot help it. He has stated that there is no real relation between the morphologic changes in the glomeruli and those of the tubules. I should like to know what Dr. Oliver's opinion is of the contradiction in this demonstrated fact with the widely accepted opinion that the blood supply of the tubules to a large extent has to go through the glomerular loops. Often in cases of glomerulonephritis one sees most of the glomerular loops almost empty, without any blood, and, nevertheless, sees little change in the tubules. I have never been able to reconcile this with the absence of a nonglomerular blood supply to the tubules as stressed by most pathologists and anatomists.

Dr Oliver has asked a question concerning the word "metallaxis" I think this word should be pronounced as the more familiar term "parallaxis" is pronounced Both words are derived from the Greek *allatto* (αλλαττω), which means to change It is a good term and might be used not only for the kidney and its vessels Certain structural changes that take place in the chronically congested liver, for instance, could be termed metallaxis No other term in English nomenclature is available for this change, the German authors call it *Umbau*

JEAN R OLIVER In answer to Dr Klemperer's question, I might say that my co-workers and I did not dissect any very small amyloid kidneys We never had any kidney to which we really wanted to give the name "contracted kidney", by that I mean a kidney 3 or 4 cm in longest dimension All of these specimens were taken from kidneys of about normal size Most were somewhat under normal, but they were not severely contracted They were all kidneys, for example, which one could easily determine grossly to be amyloid kidneys My answer to his question cannot be very direct

As to whether the inflammation which is found in these sections might be pre-existent inflammation, I do not know any way to deny it As Dr Polaves points out, one finds such inflammation very commonly in all sorts of kidneys, but I don't think that means it has nothing to do with the parenchymal deformity that one finds in the same kidneys, because that also is very common I do not think there is anything specific or peculiar or primary or unique in the inflammatory reaction one finds in the interstitial tissue of the amyloid kidney, nevertheless that reaction is there, and I cannot see how it can help but produce changes in the parenchymal structures

The matter of the blood flowing through the glomeruli before reaching the tubules, which Dr Plaut brought up, is one of those considerations that have been disturbing, as he says, to pathologists They look at the glomeruli and cannot see any patent blood vessels in them, and yet they have been taught that this is the only way that blood can get to the tubules I have no doubt that in abnormal kidneys there are many ways that blood can get to the tubules other than through the glomeruli Dr Plaut mentions the Ludwig vessels There are also direct branches from the interlobular artery, and there are arteriovenous connections or anastomoses that allow the blood to flow from the artery into the vein, then backward out around the tubules It never seemed to me that this last way was a very efficient way of irrigating the kidney, but it might help When one looks at a section and sees occlusion of glomerular capillaries, one should remember that one is looking at only a few and that the condition of the capillaries varies in normal kidneys, some being patent and some shut It is therefore difficult to estimate the state of the circulation of the kidney by looking at a few sections of a few glomeruli

I am thankful to Dr Plaut for the philologic discussion, it never occurred to me that the word "metallaxis" was similar to "parallaxis", of course, it is

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## CHICAGO PATHOLOGICAL SOCIETY

S A LEVINSON, *President*

EDWIN F HIRSCH, *Secretary*

*Regular Monthly Meeting, Dec 11, 1939*

### Experimental Retinoblastoma ARTHUR WEIL and L L MAYER

During experiments to produce retinal tumors by injecting carcinogenic chemicals into the eyes of white rats, the solvents lard and olive oil, alone, were found capable of stimulating the proliferation of retinal cells Experiments were then made on white rats by injecting superheated lard into the vitreous During the early stages a mass of granulation tissue developed The ganglion cells at

this stage were increased in number and size. Three months after the injection of lard an increase in the width of the retina to two or three times its original width was observed, accompanied by an increase in the number and the size of the cells of the inner nuclear layer. At this stage the cytoarchitecture of the retina was preserved. Gradually the outer reticular layer was narrowed by the proliferation of the cells of both nuclear layers. Many rosettes were present, the small nuclei surrounding them had active mitosis. During the seventh month the newly formed tissue completely filled the chamber and invaded the optic nerve. The choroid was vascularized at this stage, but apparently there was no proliferation of the cells in the pigmented layer. The ciliary body was markedly hypertrophied.

#### DISCUSSION

**PAUL A. STEINER.** On what basis do you distinguish between neoplasia and hyperplasia? The characteristics of neoplasia include the penetration of the eyeball by the growth, the production of metastases and the ability of transplanted tissues to continue growth. Did the superheated lard become fluorescent and was the residual material saponifiable?

**ARTHUR WEIL.** In hyperplasia the normal structure of an organ is preserved, and the normal tissue cells are increased in number. Applying this definition, I may say that up to the fourth month following injection of lard into the corpus vitreum the retina shows hyperplasia. Later its cytoarchitecture is destroyed. At first the outer reticular layer is invaded by newly formed cells, but about the sixth month there is invasion of the inner reticular layer, which, with its dense layers of nerve fibers, forms a barrier hard to penetrate. Finally the entire posterior chamber is filled by the tumor. The optic nerve, too, is invaded. The retinal cells change their histologic appearance. While normally the nuclei of the inner nuclear layer measure from 5 to 6 microns and are hyperchromatic, the newly formed cells have large vesicular nuclei, 8 to 9 microns in diameter, and contain a few coarse granules of chromatin. If one adds to the proliferating growth the destruction of the normal structure, the invasion of the optic nerve and the active nuclear division observed during the sixth month, there should be no doubt that there is a neoplasia of the retina.

In human retinoblastoma, metastasis to other organs occurs rarely, but invasion of the optic nerve and finally of the brain is seen during the late stages. Fractionation of the lard was attempted. Separate fractions of the distillate up to 250 C. in vacuo were injected, without success. Saponification with potassium hydroxide destroyed the neoplastic properties. Trauma of the retina alone and injections of foreign bodies, such as a charcoal suspension, colloidal silver and cholesterol emulsions, did not lead to hyperplasia or tumor formation.

#### Enteric Intramural Herniation Following Colostomy **GEORGE J. RUKSTINAT,** **CHESTER B. THRIFT and FRANK M. SYLVESTER**

The factors predisposing to prolapse of the large bowel after colostomy are the length of the mesentery, which permits intestinal evagination, the weakness of the abdominal wall as a result of improper technic or of atrophy as a sequel to suppuration, and altered innervation of the intestine. The direct cause of the prolapse may be active (e. g., a violent straining effort at stool) or passive (e. g., the aspiration action of a cup type of apparatus).

The anatomic forms of prolapse are simple, involving only the mucosa of the colon, total, involving the entire wall of the colon, hernia of a small bowel into the colon, strangulated. This report concerns elements of all the foregoing types and, in addition, the complications of extensive herniation of the small bowel between the fascial layers of the abdominal wall and a fistulous passage from the urinary bladder to the distal part of the colostomy loop.

A white man aged 76 complained of scalding urine, looseness of the bowels and occasional drops of blood in the stools, which came from the proximal opening of his colostomy. Simultaneously with the passage of fecal matter from the

proximal colostomy opening, urine was discharged from the distal opening. Twenty-four years before the onset of these complaints he had not defecated for nine days and had had surgical intervention for a supposed tumor of the bowel. A pelvic mass, palpated at operation at that time, was not removed, but a permanent colostomy was established above the involved region. Thirteen years later a protruding portion of bowel was resected from the upper colostomy opening. Six years later diabetes mellitus developed, which was controlled by dietary treatment. On admission to the Loretto Hospital with the complaints stated, the patient had a pendulous abdomen from which the descending colon protruded 32.5 cm from the proximal colostomy opening. When the bladder filled, urine passed from the distal opening. There was a large indurated mass in the pubic region, and another was palpated near the pylorus. Three weeks later the patient was critically ill and dehydrated. He had not defecated in forty-eight hours.

He had cramping pain in the upper part of the abdomen, and a large firm mass occupied the left hypochondrium to the umbilicus. Death followed several days after ileocolic enterostomy.

The pertinent portions of the anatomic diagnosis were: hypostatic bronchopneumonia, primary carcinoma of the tail of the pancreas, generalized carcinoma metastases of the liver, peritoneum and biliary lymph glands, marked extrusion of the proximal colostomy loop, and herniation of the small bowel intramurally into the abdominal wall about the colostomy and into the protruding colostomy loop, vesicocolic fistula. The lumen of the distal portion of the colon and rectum was only 3 to 5 mm in diameter, the wall was 8 mm thick, indurated and pale gray-white. The fistulous tract was surrounded by connective tissue and small nests of lymphocytes. The absence of carcinoma from this part of the bowel suggests that the condition for which the original colostomy was done was probably a diverticulum of the colon. Inflammation of the wall of the diverticulum led to obstructions of the bowel, adhesions between the bowel and the bladder and, eventually, perforation of the bladder.

#### **Hypertension (Goldblatt) and Unilateral Malignant Nephrosclerosis** OTTO SAPHIR

Two patients had severe arterial hypertension secondary to unilateral renal vascular stenosis with consequent ischemia of one kidney. Both of these had at autopsy unilateral malignant nephrosclerosis. This unique finding could be explained by recent experimental evidence. Goldblatt concluded from his experimental studies, primarily concerned with the production of arterial hypertension by clamping the renal arteries in dogs, that both hypertension and renal insufficiency are the minimal prerequisites for the induction of arteriolonecrosis, for in the absence of either of these factors no necrotizing changes are observed. In each case reported here severe arterial hypertension was brought about by renal arterial changes resulting in ischemia of one kidney. Renal excretory insufficiency subsequently developed in both cases. It was precipitated in one instance by the onset of congestive heart failure and in the other by the development of acute ascending pyelonephritis in the kidney opposite the ischemic one. Because of the presence of the severe arterial hypertension and excretory renal insufficiency, the arterioles in the contralateral kidneys, with patent vascular system, showed necrotic changes, and these kidneys presented the typical picture of malignant nephrosclerosis. The arterioles in the ischemic kidneys revealed no necrotic changes because the stenosis of the renal and intrarenal arteries militated against the presence of severe hypertension within the arterioles. Thus, the genesis of the malignant nephrosclerosis in these cases is exactly similar to that of the arteriolonecrotic changes produced experimentally by Goldblatt.

#### **Dystrophic Calcification of the Myocardium with Glomerulonephritis** R. M. BOLMAN

This article will appear in full in a later issue of the ARCHIVES

## Book Reviews

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**Virus and Rickettsial Diseases, with Especial Consideration of Their Public Health Significance** A Symposium held at the Harvard School of Public Health, June 12-17, 1939 Cloth Pp 907, illustrated Price, \$6 50 Cambridge, Mass Harvard University Press, 1940

After twenty years of intensive investigation the world over, there has accumulated such a wealth of detailed knowledge concerning the filtrable viruses and the diseases they cause that need has arisen for a comprehensive and critical orientation of the subject matter and theory both for the investigator himself and for students of medicine and allied sciences. In response to this want, several excellent treatises have appeared in recent months, prepared by individual authors or by groups of authors, themselves authorities within their special fields. The symposium held at the Harvard School of Public Health in June 1939 represented a response to the aforementioned need for a discussion and general consideration of virus diseases especially from the standpoint of public health, and the volume under review is the published product of that conference. A general survey of rickettsial diseases, their clinical features, diagnosis, classification, epidemiologic features and immunity, was included because of certain analogies obtaining between them and the filtrable virus groups that have caused some confusion.

It is significant of the present absorbing interest in the virus field that most of the twenty-four authors of the thirty-four papers comprising the volume are investigators actively engaged in research on certain phases of the subject they present, and at a single university or in its environment.

Each paper is a review of important advances in knowledge and theory related to the subject matter and is followed by a reasonably full bibliography. The rather comprehensive introductory paper deals with "Epidemiologic Problems in Virus Diseases" and serves both to introduce the book and to emphasize the public health aspects of the diseases under consideration. This is followed by an adequate consideration in three papers of physical and chemical properties, immunologic aspects and insect vectors of viruses and their diseases.

Subsequent papers deal almost exclusively with the more important virus diseases of man, namely, variola-vaccinia, measles, mumps, dengue fever, venereal lymphogranuloma, influenza, psittacosis, poliomyelitis, rabies, equine encephalomyelitis, lymphocytic choriomeningitis, louping ill and yellow fever, in the order mentioned.

The several contributions are for the most part of excellent critical quality, though a few are rather summary. Certain contributors are, as might be expected, more at home when dealing with special phases of individual problems than in a general consideration of theory and outlook. The subject of viruses is perhaps not yet quite ripe for indulgence in generalization, but there is noticeable a certain haziness of ideas as well as looseness in the use of terms that even now should be applied more precisely. It seems to this reviewer, for example, no longer justifiable to use the term "ultramicroscopic viruses" with comprehensive significance when it is well known that the elementary bodies of such virus lesions as those of fowlpox, molluscum contagiosum and others can be very easily seen in fresh preparations through a high power oil immersion system and that these structures might well be the formal representations of the respective active agents so far as observers know at present. Furthermore, while the authors generally accept the point of view that viruses invade and undergo multiplication inside of cells as necessary conditions to infection, there seems at times to be a lack of critical appreciation of the desirability of more precise knowledge respecting this cytotropic property, especially from the standpoint of clarity in thinking and in



investigation of pathogenesis. For example, in the discussion of classification of virus diseases of the central nervous system (p 617) some point is made to criticize a rather loose use of the adjective "neurotropic," yet the author himself indulges with unconscious ease and evident innocence in the acceptance and use of such indefinite and confusing adjectives as "pantropic" and "viscrotropic" as bases for orderly arrangement. It is refreshing to see one author (p 720) take exception to such confusion, although nowhere in the book is the basic hypothesis of cytotropism of viruses clearly and understandingly considered.

The volume as a whole is a timely one and will be of great help to students, teachers and investigators of disease generally.

**Diseases of the Gallbladder and Bile Ducts** Waltman Walters, B S, M D, M S, Sc D, and Albert M Snell, B S, M D, M S Pp 645 Price \$10 Philadelphia W B Saunders Company, 1940

One of the important advances in modern medicine has been the development of reviews of subject material in monograph form. The reviews in the basal sciences have for the most part been characterized by a greater depth of scholarship than is apparent in those in the clinical fields of medicine. This book by Walters and Snell, with eight of their associates at the Mayo Clinic, can be compared favorably with the best of the reviews. While little appears that is new, the authors have gathered together a great deal of published data bearing on the biliary tract from many angles, and the compilation is equal to the high standards which one has come to associate with the work of the Rochester group. The bibliography is superb and is a good point of reference for wider studies.

The anatomy, physiology and pathology of the biliary tract are reviewed, and then the clinical aspects of diseases in this system are considered, followed by details of medical and surgical treatment. The surgical aspects of the field are considered in the greatest detail. The book is well illustrated with 195 figures.

In parts the book is repetitious, and the etiology of gallstones and the pathology of cholecystitis are discussed in several scattered places. A second edition would profit by more efficient arrangement and condensation. Several contradictory statements are made at times by the same author. Thus, one reads "A review of this large series of surgically removed gallbladders suggests that gallstones, regardless of whether they are metabolic or bacterial in origin, constitute the greatest menace to the welfare of their hosts" (p 86), and "Judging from the fact that according to postmortem records a relatively high percentage of individuals have stones and the fact that so very few people die directly from gallstones in the gallbladder it might be suggested from this material that removal of gallstones simply because they are found roentgenoscopically is not justified, especially if their host has no incapacitating signs and symptoms" (p 87).

The chemical nomenclature at times is loose, thus, sodium hypochlorite is referred to as "chlorinated soda" (pp 45 and 47), and such statements as "white stones are largely composed of calcium" (p 84) are obviously rich in misnomers. One also could wish for a treatment of precipitation in bile from the standpoint of physical chemistry.

The book is highly recommended to those especially interested in the biliary tract.

## SO-CALLED MIXED TUMORS OF THE MAMMARY GLAND OF DOG AND MAN

WITH SPECIAL REFERENCE TO THE GENERAL PROBLEM OF CARTILAGE AND BONE FORMATION

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NEW YORK

A striking discrepancy in comparative pathology that has been recognized but that has remained unexplained for more than a century is the remarkably high incidence of cartilage and bone in tumors of the mammary glands of dogs as contrasted with man<sup>1</sup>. Precise statistics on histologically studied material are scarcely available. Nieberle<sup>2</sup> reported that 75 per cent of the neoplasms of breasts of dogs are mixed tumors in which cartilage is "frequently" present and bone somewhat less frequently. Of the 28 tumors described by Glendinning,<sup>1g</sup> cartilage or bone was found in 5, or approximately 18 per cent. Of 13 mammary tumors examined in this laboratory, cartilage or bone was present in 4, or about 30 per cent. On the other hand, mammary tumors containing cartilage or bone are rare in man (Wilms<sup>3</sup>, Thinnes<sup>4</sup>, Sehr<sup>1h</sup>). Gross<sup>5</sup> stated in his monograph that he had seen only 1 case of

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From the Laboratories of the Mount Sinai Hospital and the New York Zoological Park

1 (a) Virchow, R. *Die krankhaften Geschwulste*, Berlin, A. Hirschwald, 1863, vol 1, pp 481, 484, 507, 520 and 524 (b) Billroth, T. *Virchows Arch f path Anat* **18** 51, 1860 (c) Labbé, L., and Coyne, P. *Traité des tumeurs bénignes du sein*, Paris, G. Masson, 1876, pp 330-331 (d) Cornil, V., and Petit, G. *Bull Soc anat de Paris* **80** 23, 1905 (e) Kitt, T. *Text-Book of Comparative General Pathology*, translated by W. W. Cadbury, Chicago, Chicago Medical Book Company, 1906 (f) Frei, W. E., in Joest, E. *Handbuch der speziellen pathologischen Anatomie der Haustiere*, Berlin, R. Schoetz, 1925, vol 4, p 74 (g) Glendinning, B. *Arch Middlesex Hosp* **19** 198, 1910 (h) Sehr, E. *Beitr z klin Chr* **55** 574, 1907 (i) Ewing, J. *Neoplastic Diseases*, ed 2, Philadelphia, W. B. Saunders Company, 1928, pp 538, 773-775 and 1018-1019

2 Nieberle, K. *Ztschr f Krebsforsch* **39** 113, 1933

3 Wilms, M. *Die Mischgeschwulste III Mischgeschwulste der Brustdrüse*, Leipzig, A. Georgi, 1902

4 Thinnes, H. *Virchows Arch f path Anat* **264** 150, 1927

5 Gross, S. W. *A Practical Treatise on Tumors of the Mammary Gland*, New York, D. Appleton and Company, 1880, p 53

ossification of the breast, that of an ossifying fibroma Labbé and Coyne<sup>1c</sup> stated that they had never observed such tumors and insisted that those described in the literature (up to 1876) were all disputable Cheattle and Cutler<sup>6</sup> mentioned that in their entire experience they had seen only 2 examples of cartilage in the human breast Heuter and Karrenstein<sup>7</sup> in 1906 collected 5 instances of chondrosarcoma and 3 of osteosarcoma from the literature In 1927 Thimmes<sup>4</sup> recognized the cases of only eight authors as definitely instances of osteochondrosarcoma Gomori<sup>8</sup> in 1936 was able to collect from the literature only 6 instances of mammary tumor with "well-developed bone tissue as a characteristic element" Although the actual number of such tumors is undoubtedly greater than the citations indicate, nevertheless, their relative rarity remains unquestioned

Mammary tumors containing cartilage and bone are uncommon also in the horse, cow, goat and sheep (Frei<sup>1f</sup>) In reports of observations made on hundreds of spontaneous mammary tumors of mice by Haddow<sup>9</sup> and Haaland,<sup>10</sup> no mention is made of the presence of cartilage or bone Murray<sup>11</sup> recorded a single cartilaginous tumor among 87 such growths However, in a series of 87 spontaneous sarcomas of various organs of mice, Slye, Holmes and Wells<sup>12</sup> found 4 "subcutaneous or mammary" tumors containing cartilage In experiments with a transplantable carcinoma of mice, which is described as having undergone sarcomatous transformation, Woglom<sup>13</sup> observed the presence of cartilage in 3 of the 4 mammary tumors in the second generation In regard to rats, Bullock and Curtis<sup>14</sup> found no cartilage or bone in 94 spontaneous tumors It may be safely concluded, therefore, that the incidence of cartilage and bone in tumors of mammary glands is peculiarly high in dogs

There are, to be sure, other discrepancies in comparative oncology which lack adequate explanations It is well known, for example, that tumors of the lip, esophagus, stomach (except the series of tumors of rats reported by Bullock and Curtis) and rectum are relatively rare in animals<sup>15</sup> This is in strong contrast to their frequency in man

6 Cheattle, L., and Cutler, M. Tumors of the Breast, Philadelphia, J B Lippincott Company, 1932, p 485

7 Heuter, C., and Karrenstein. Virchows Arch f path Anat **183** 495, 1906

8 Gomori, G. Am J Surg **33** 150, 1936

9 Haddow, A. J Path & Bact **47** 553, 1938

10 Haaland, M. Scient Rep Invest Imp Cancer Research Fund **4** 1, 1911

11 Murray, J A. Scient Rep Invest Imp Cancer Research Fund **3** 41, 1908

12 Slye, M., Holmes, H F., and Wells, H G. J Cancer Research **2** 1, 1917

13 Woglom, W H. J Cancer Research **3** 47, 1918

14 Bullock, F D., and Curtis, M R. J Cancer Research **14** 11, 1930

15 Dobberstein, J. Virchows Arch f path Anat **302** 1, 1938 Wells, H G., Slye, M., and Holmes, H F. Am J Cancer **33** 223, 1938 Moschkowitz, L., and Sprinz, H. *ibid* **38** 271, 1940 Kitt<sup>1e</sup> Slye, Holmes and Wells<sup>12</sup>

It is felt that in the explanation of such differences there must lie facts of fundamental oncologic and possible histologic importance, particularly as relates to the much controverted subjects of chondrogenesis and osteogenesis. It was therefore decided to reconsider the subject when the opportunity for studying these mammary tumors presented itself. Those who have written on the presence of cartilage and bone in the breast have generally been concerned not with the reasons for the high incidence in dogs but with the genesis of these tissues. The purpose of this communication is therefore twofold:

- 1 To attempt to trace the genesis of cartilage and bone in these tumors

- 2 To endeavor to isolate those factors peculiar to the dog which may be responsible for the unique incidence of cartilage and bone-containing mammary tumors in that species

#### MATERIAL AND METHODS

The material to be considered includes 4 mammary tumors from dogs and 1 such tumor from a woman. In addition, because of certain features of the genesis of the cartilage and bone, 5 cases of gelatinous carcinoma of the human female breast are briefly analyzed.

The following stains were used: hematoxylin and eosin, Weigert and Van Gieson, Mallory's aniline blue, Best's carmine, Mayer's mucicarmine, Bielschowsky's silver, toluidine blue, iron and sudan. The tissues were subjected to pancreatic and tryptic digestion for aid in the differentiation of collagen and reticulum from mucus and fibrin.

#### TUMOR FROM DOG 322

*Macroscopic Observations*—The specimen was removed surgically from a 7½ year old female Russian wolfhound. The tumor consists of a roughly spherical, moderately firm mass about the size of a grapefruit, measuring 14 cm in diameter. The mass is covered by easily cleaved, apparently uninvolved skin except along the resected surface, which is formed by tumor. The sectioned surfaces present a variegated appearance. They are for the most part grayish tan mottled irregularly with pale to slate gray tissue, in which are scattered small areas of deep brown pigment. Numerous cysts measuring up to 3 cm in diameter are present. On close examination, one is able to distinguish several small gray irregular foci, which have the consistency of cartilage.

*Microscopic Observations*—The sections present a very heterogeneous picture. The tumor is composed, for the most part, of irregular nodules of glands surrounded by varying widths of fibrous tissue, which occasionally appear compressed into pseudocapsules. In places, this stroma is invaded, with consequent disappearance of part of the capsule and coalescence of adjacent nodules. Scattered in the stroma are several small abscesses, as well as numerous macrophages filled with fat, carminophilic droplets and iron pigment.

The glands are highly irregular in size and shape. Some are filled with a homogeneous, apparently albuminous eosinophilic secretion, which does not take the carmine or toluidine blue stain and in which macrophages and polymorphonuclear leukocytes are frequent. Many of the glands are lined by stratified layers of

columnar cells with large, moderately vesicular nuclei showing little atypism. One is frequently able to make out an incomplete layer of "basket" or myo-epithelial cells along the outer border of the glandular epithelium, just adjacent to the basement membrane. The epithelium of many of the glands forms stalklike invaginations with delicate central fibrous cores. Some of the glands are partially to completely filled with compact cellular masses.

Of particular interest are those glands whose innermost layer of epithelium is just beginning to loosen and to separate from the adjacent cells. In these, one frequently sees carminophilic droplets, both in the cytoplasm and in the intercellular fluid. The intercellular spaces become progressively wider, and the

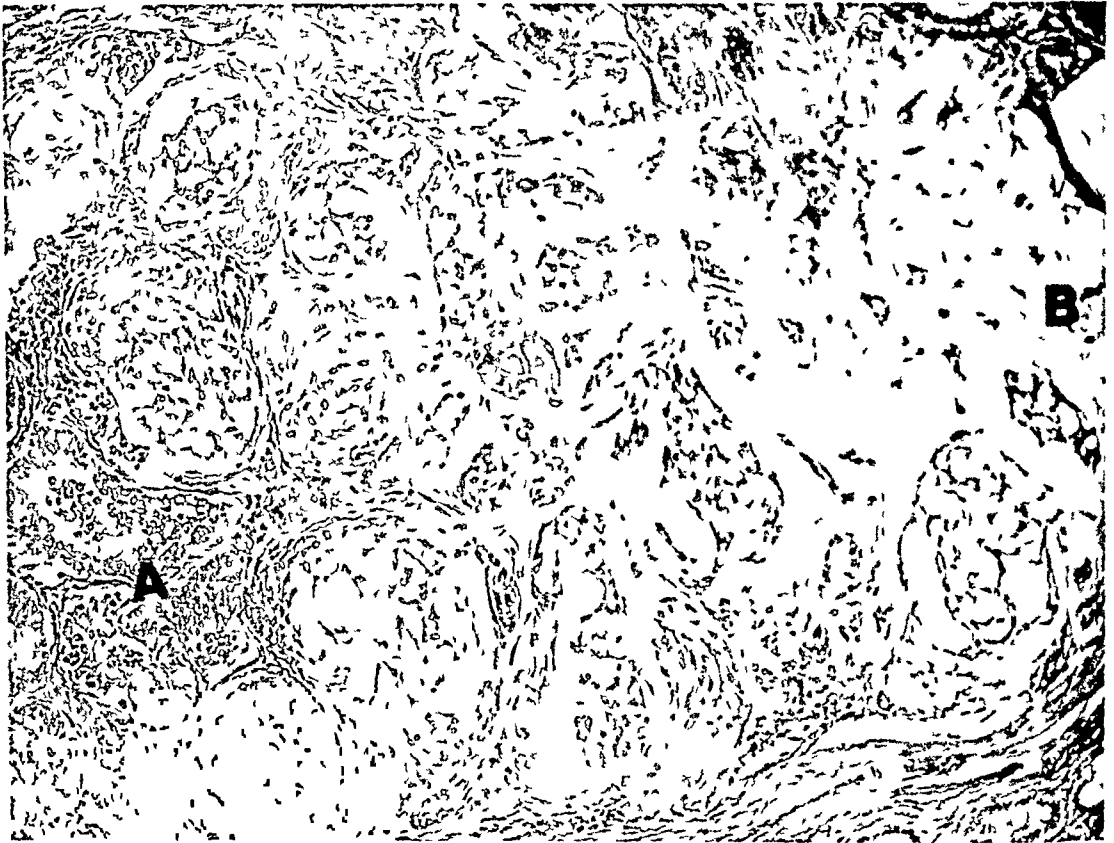


Fig 1 (dog 322) —This section illustrates several of the early transitional stages in the development of cartilage from epithelium. Note the gradual separation of the *adult* epithelium first into a retiform pattern and finally into completely isolated single cells, pairs or small clumps. As with normal epiphyseal cartilage, both the matrix and the cells exhibit an increase in carminophilia, as well as metachromasia with toluidine blue, with the progression of the transition from the compact epithelium at *A* to the isolated cells at *B*. Hematoxylin and eosin.

cells appear pushed farther and farther apart, with resultant gradual encroachment on, and finally obliteration of, the lumen of the gland. In short, what was once stratified epithelium cuffing a lumen has become a delicate laciform tracery (fig 1). Where the intercellular fluid has accumulated in sufficient quantity, this reticular network originating from the glandular epithelial lining is forced apart, and the cells become isolated in a mucoid matrix (figs 2, 3 and 4). In the

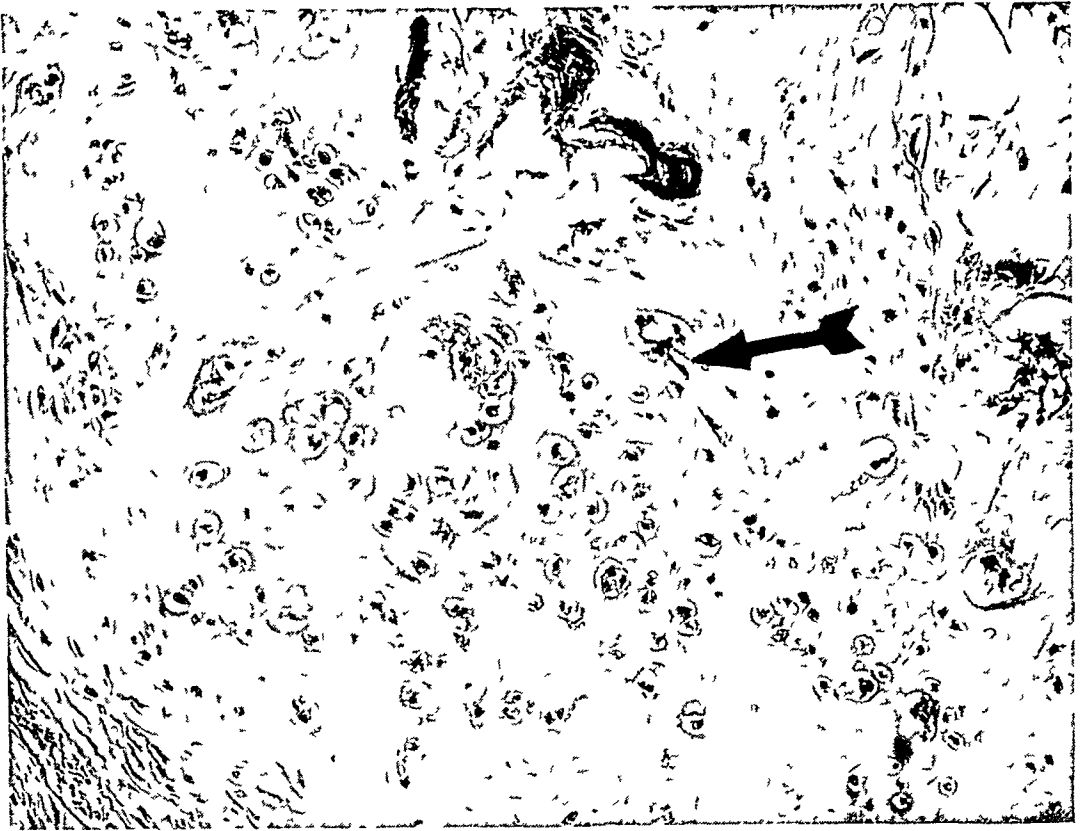


Fig 2 (dog 322) —This field demonstrates clearly the mechanism of isolation of the originally adult acinar epithelium within the mucocollagenous matrix and the end stages of the transformation into cartilage. The arrow points to a cell about to be enclosed by matrix. Hematoxylin and eosin

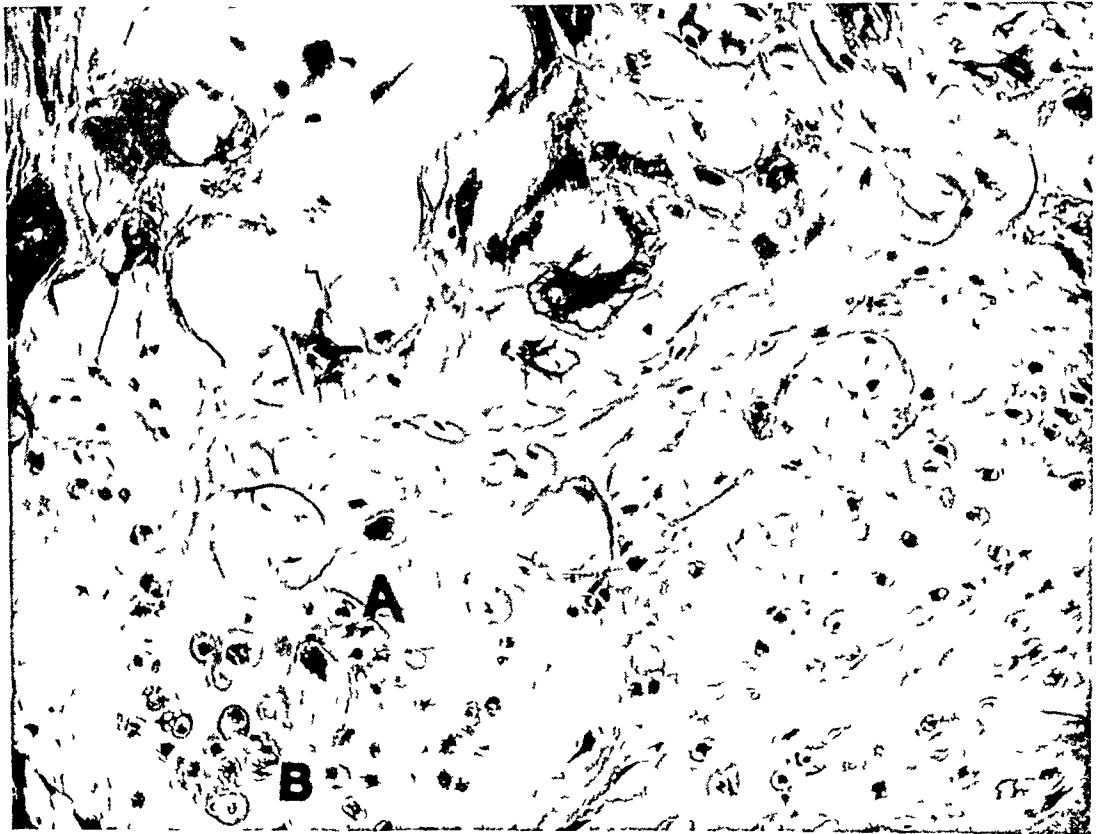


Fig 3 (dog 322) —This section illustrates the transition of epithelium into cartilage by the process of disruption of the acinar epithelium (A) within the mucocollagenous matrix. The cells in the end stage (B) are indistinguishable from chondrocytes. Hematoxylin and eosin

meantime the cells have become spindle shaped and often are filled with carminophilic droplets. The matrix in which they are embedded generally stains only faint pink with carmine or mucicarmine but manifest definite metachromasia with toluidine blue. The basket cells appear to suffer the same fate as the remainder of the epithelial lining, and they, too, come to lie loose in the fluid matrix. However, one does not observe selective proliferation of these cells. The normal ratio between these myoepithelial cells and the remaining epithelial cells is at most maintained and is frequently diminished.

Scattered through the matrix are partially fragmented fibers which stain pink to red with acid fuchsin, pale blue to deep blue with aniline blue and yellowish brown to black with silver and which for the most part resist tryptic and pancreatic

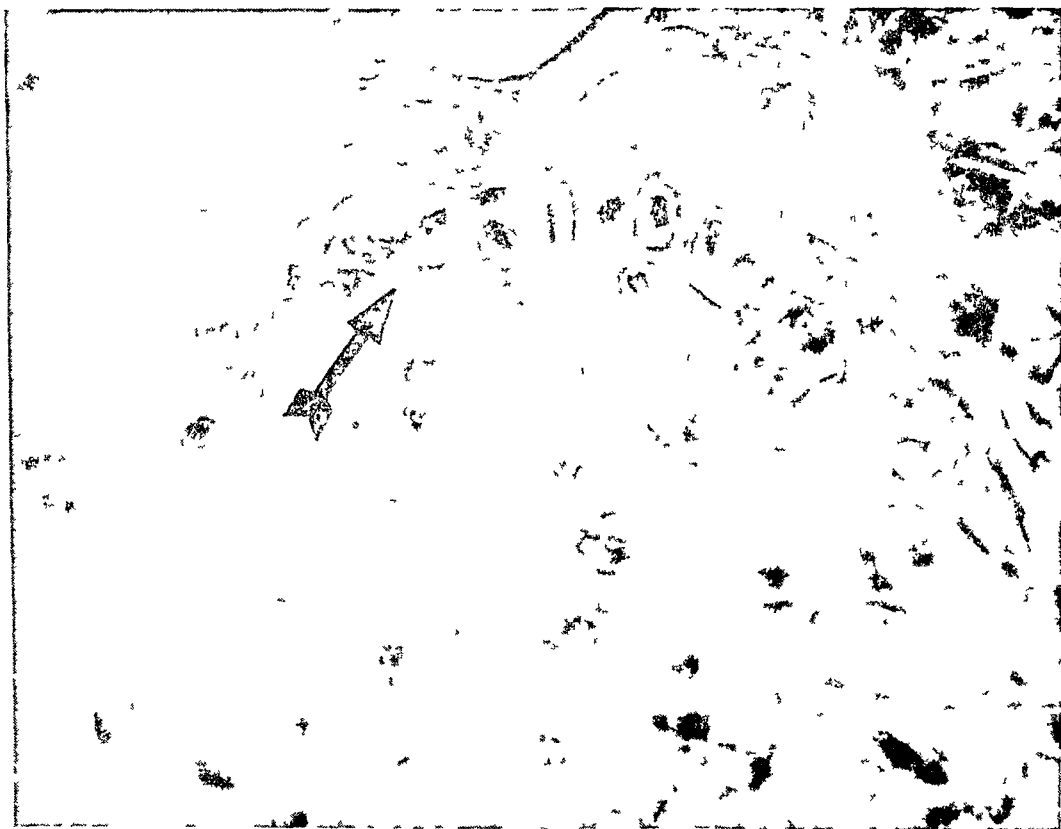


Fig 4 (dog 322) —High power magnification of a cartilaginous focus, illustrating the mechanism of isolation of epithelial cells by the matrix. The arrow points to a disrupting clump of acinar cells. Bielschowsky's silver stain.

digestion. They are therefore identifiable as collagenous and reticulum fibers. The source of the reticulum fibers appears most likely to be the argyrophilic basement membrane of the disrupted acini. Part of the collagenous tissue obviously arises from the surrounding stroma, since definite continuity is observed in many places. Collagenous fibers are seen also directly within the mucus, however, as if there has occurred collagenous transformation of the mucus, or, at any rate, partial replacement of the latter by collagenous fibers.

In those areas where the metachromasia is most pronounced, there are isolated epithelial cells partially surrounded by coves of matrix or actually completely enclosed in lacunae (figs 2, 3 and 4). The cytoplasm of these cells contains

abundant carminophilic granules, as does the cytoplasm of chondrocytes. The cells are anchored to the lacunar walls by cytoplasmic strands, as if the remainder of the cell had shrunk. In some of the lacunae one sees, just inside of their capsules, granular eosinophilic material, which is found commonly in normal cartilage. Small droplets of fat are seen in some of the cells. In general, the nuclei appear viable, although here and there are imprisoned degenerating epithelial cells. Such foci are histologically indistinguishable from true hyaline cartilage. It is noted that the more closely these various portions of the tumor approach the histologic pattern of cartilage, the more carminophilic and metachromatic does

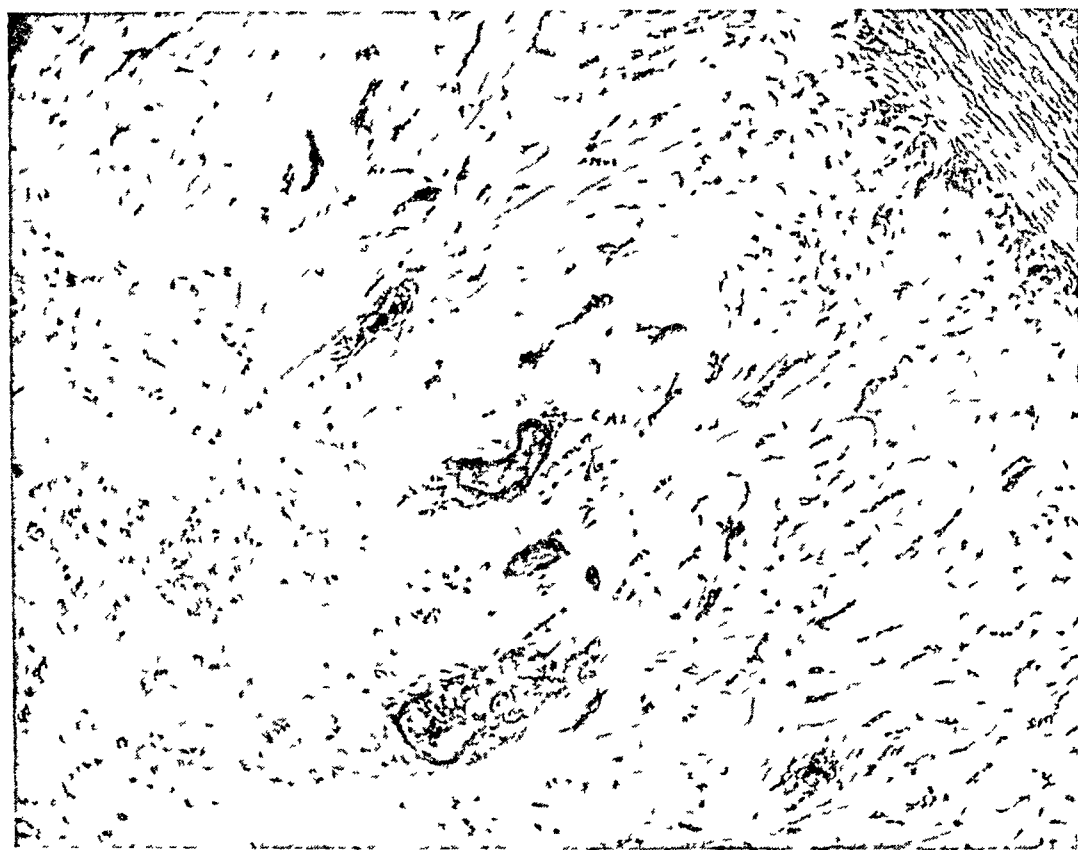


Fig 5 (dog 322) —Note transition from right to left of epithelium to early cartilage by the process of dispersion and isolation of the epithelial cells in an increasingly carminophilic and metachromatic matrix. Hematoxylin and eosin.

the matrix become. In parallel manner, the enclosed cells stain progressively more deeply with carmine as the tissue takes on the appearance of cartilage.

*Diagnosis*—The tumor is a myxochondroadenocarcinoma.

*Comment*—This tumor is interpreted as representing an adenocarcinoma with foci of transformation of epithelial cells into myxomatous tissue and cartilage. In this process there has occurred a replacement of a mucoid fluid by collagenous tissue. It is impossible to be certain whether or not this replacement signifies direct conversion of mucus into collagen—a phenomenon acknowledged by Ehrlich,<sup>16</sup>

<sup>16</sup> Ehrlich, E. *Beitr z klin Chir* 51 368, 1906.



von Ebner,<sup>17</sup> Ricker and Schwalb<sup>18</sup> and Ewing<sup>11</sup>—or derivation from preexisting collagen. It is significant that the progressively increasing carminophilia of the transition stages of the "epithelial cartilage" parallels that observed in the developing epiphyseal cartilage (Harris<sup>19</sup>).

#### TUMOR FROM DOG 701

The specimen was removed from the mammary region of a stray adult female bitch of unknown age.

*Macroscopic Observations*—The specimen consists of a spherical, moderately firm tumor mass about the size of a walnut (3 cm in diameter), to which the

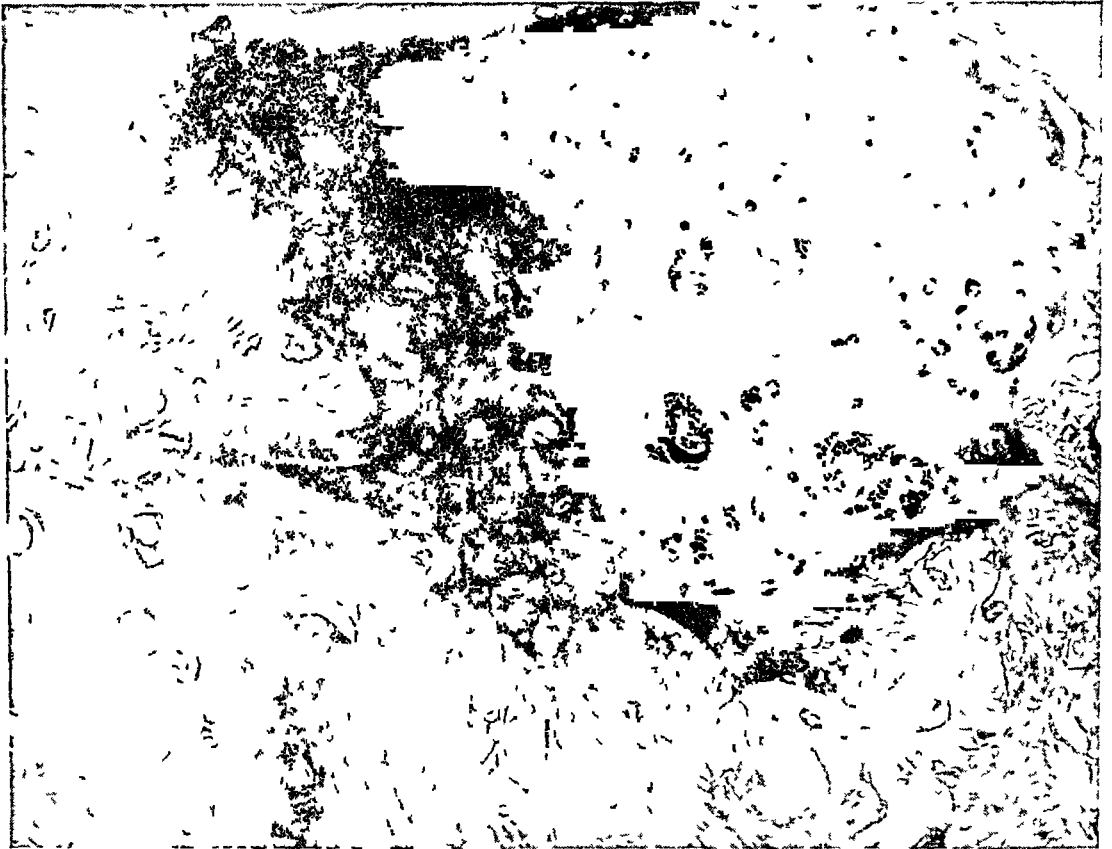


Fig 6 (dog 322)—Nonencapsulated foci of cartilage. Note the increasing metachromasia as the epithelial tissue and its matrix progressively acquire the characteristics of cartilage. Toluidine blue.

overlying skin is adherent. The sectioned surface is pale purple gray mottled with bright yellow, 2 to 3 mm flecks. Several areas varying from 4 to 8 mm in diameter are light gray and have the consistency of cartilage. Two large cysts, measuring 12 by 0.5 and 15 by 0.8 cm, are present at the periphery.

*Microscopic Observations*—Sections show the tissue to be composed of glands with marked variation in size and shape. Several areas are necrotic, hemorrhagic

<sup>17</sup> von Ebner. *Ztschr f Zool* **62**: 469, 1896.

<sup>18</sup> Ricker, G., and Schwalb, J. *Die Geschwulste der Hautdrusen*, Berlin, S. Karger, 1914, pp 210-224.

<sup>19</sup> Harris, H. A. *Nature*, London **130**: 996, 1932.

and loosely infiltrated with polymorphonuclear leukocytes. Irregular deposits of calcium are present in some of these necrotic areas. The glands are lined by columnar or polygonal cells containing large hyperchromatic nuclei with one or more prominent nucleoli. Mitotic figures are abundant. In many glands the epithelium is stratified or papillary and frequently fills the lumens. In several areas the epithelium is reticulated so as to simulate parts of the tumor of dog 322. In addition to the reticulated epithelium, the cells of which are separated by a homogeneous fluid, there are found glands in various stages of disruption lying loose in the eosinophilic, partially metachromatic (toluidine blue) matrix. As in the previous case, there are a few areas where one can easily follow the isolation of these cells within the mucoid matrix. There are, in addition, however, isolated spindle cells with vesicular nuclei which strongly resemble fibroblasts. The cytoplasm of both these types of cells, as they lie isolated in the matrix, is vacuolated and markedly carminophilic. Some of the cells lie in lacunae surrounded by "capsules," to which shreds of cytoplasm are adherent, as if the irregular spaces within the lacunae are the consequence of the cytoplasmic vacuolation. About some of the lacunae harboring intact cells, as well as near several clumps of cells not in lacunae but within matrix, there is a sprinkling of fine calcareous granules. An occasional nucleus in these regions is intensely basophilic, as if calcified. In those areas where chondrification has progressed, one observes an increasing affinity of the matrix for aniline blue and carmine, as well as distinct bluish (Mallory's aniline blue) bundles of collagenous fibers.

*Diagnosis*—The tumor is a myxochondroadenocarcinoma.

*Comment*—The cells taking part in the chondrification appear to be both epithelial and mesenchymal. Collagenous fibers within the matrix are obvious. It is interesting to note the affinity of the matrix for calcium as shown by the presence of the calcareous granules, particularly about lacunae containing intact cells. The affinity of the perilacunar matrix for calcium is exhibited generally in the calcification of ordinary osteoid tissue (Dibbelt<sup>20</sup>).

#### TUMOR FROM DOG 112

The animal was an 11 year old female Kerry blue terrier.

*Macroscopic Observations*—The specimen consists of an oval encapsulated firm tumor mass, measuring 6 by 4 cm, removed surgically from the hindmost breast. The overlying skin is not adherent. The sectioned surface is pale purple gray mottled with yellow flecks and several small hemorrhagic areas. The consistency of the sectioned surface is similar to that of raw potato.

*Microscopic Observations*—Sections reveal the tumor to be composed principally of hyaline cartilage and osteoid tissue merging with compact nests of polygonal cells with large hyperchromatic nuclei and prominent nucleoli. Mitotic figures are numerous. The cytoplasm of these cells is relatively abundant and occasionally tapers at either pole, particularly near the periphery of the cell nests. In these regions the cells appear to merge with the surrounding scanty stroma. In other portions the cells become separated by varying amounts of a hyaline eosinophilic material. The cells in these areas lie in lacunae as the matrix apparently inspissates, acquiring a bluish tint (hematoxylin and eosin) as well as a pronounced affinity for aniline blue. The cytoplasm of these cells is vacuolated, but the nuclei appear intact. The picture in these areas is indistinguishable from that of hyaline cartilage and includes the property of marked metachromasia. The carmine stain, however,

is negative, probably owing to delayed fixation. About the areas of osteoid and osseous tissue the tumor cells are frequently aligned in a regular row so as to resemble osteoblasts. Numerous giant cells corresponding to osteoclasts are also present both at the periphery of these foci and within the cellular portions of the tumor. There are several areas of hemorrhage and necrosis with infiltration by polymorphonuclear leukocytes, lymphocytes and macrophages.

No actual gland formation is present within the tumor proper. However, at the periphery there are encapsulated nests of glands with marked papillary projections of epithelium which are not carcinomatous. Several cysts are present which are filled with an eosinophilic, nonmetachromatic secretion.

*Diagnosis*—The tumor is an osteochondrosarcoma.

*Comment*—The cells within the chondral, osteoid and osseous tissue appear to be mesenchymal tumor cells.



Fig 7 (dog 166) —Chondroadenocarcinoma of a mammary gland with stromal chondrification and ossification. Note the encapsulation and the location directly beneath the skin. Trauma to this mammary gland is obviously borne almost wholly by the tumor.

#### TUMOR FROM DOG 166

This dog was an adult bitch, the exact age is unknown.

*Macroscopic Observations*—The specimen consists of a tumor removed at autopsy from the left caudal mammary gland. It is a firm walnut-sized mass (3 by 2.5 cm), completely enclosed in a thin fibrous capsule. The cut surface presents a motley of colors, including the deep purple-brown of old hemorrhage interspersed with irregular areas of grayish white and yellowish tan. The latter areas grate as if composed of bone.

*Microscopic Observations*—Sections reveal the tumor to be composed predominantly of nests of irregularly patterned glands of varying sizes and shapes. These are lined for the most part by a single layer of columnar cells with vesicular nuclei containing prominent nucleoli. Mitotic figures are absent. Occasionally the glands are partially occluded by projections or stratifications of epithelium. Here and there the lumen of a gland is completely obliterated by epithelium, which

has become reticulated as if fluid had accumulated between the individual cells and forced them apart. These epithelial cells appear progressively to acquire a more definite spindle shape so as to simulate within their fluid matrix a myxomatous tissue. In several areas, each about the size of a low power field, the cells no longer are spindle shaped but appear to have drawn in their cytoplasmic processes and to have become isolated in a matrix. Their cytoplasm is vacuolated and slightly carminophilic. Capsular rims are present about them, thereby simulating chondral tissue. The matrix takes the Van Gieson and Mallory stains for collagen and is not digested by pancreatin. As with ordinary cartilage, the metachromasia of these portions of the tumor is intense. In other parts, irregular trabeculae of

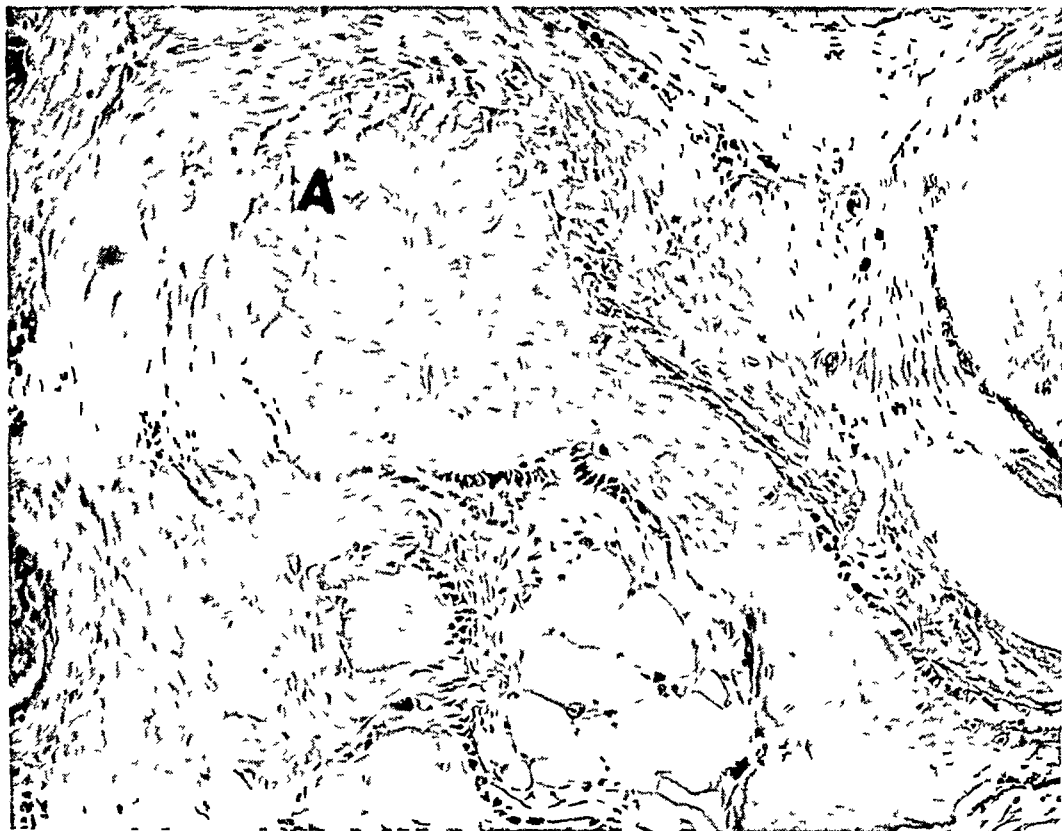


Fig 8 (dog 166) —Bony trabecula in stroma. Note at *A* the apparent advancing line of ossification and the absence of osteoblasts in this region where, according to the current concept of the secretion or transformation of the ectoplasm of osteoblasts, they ought logically to be found. Instead, this seems to represent a direct transformation of *mature* fibrous tissue with incidental inclusion of cells (osteocytes). Hematoxylin and eosin.

mature bone are found. These show typical laminations, rims of osteoblasts, scattered osteoclasts and a fatty "marrow" with small islets of cells, which are predominantly polymorphonuclear leukocytes. Some of the bony trabeculae merge with small foci of hyaline cartilage. This transition, which appears to be gradual in sections stained with hematoxylin and eosin, is seen to be sharp in those stained with toluidine blue. In these the intensely metachromatic cartilage is contrasted with the pale blue-staining adjacent bone. The osteoid and osseous tissue appears in places to be directly continuous with the collagen of the stroma as if there

had taken place a direct transformation therefrom. Furthermore, about parts of some of the trabeculae, osteoblasts are absent (figs 8 and 9). There is also much evidence of old hemorrhage in the stroma in the form of numerous collections of macrophages filled with iron pigment, as well as scattered foci showing acute and chronic inflammatory reaction.

*Diagnosis*—The tumor is a chondroadenocarcinoma with stromal chondrification and ossification.

*Comment*—This tumor is interpreted as an adenocarcinoma with cartilage derived from epithelium as well as from stroma. Bone, formed apparently by the direct ossification of the stroma, is also present.

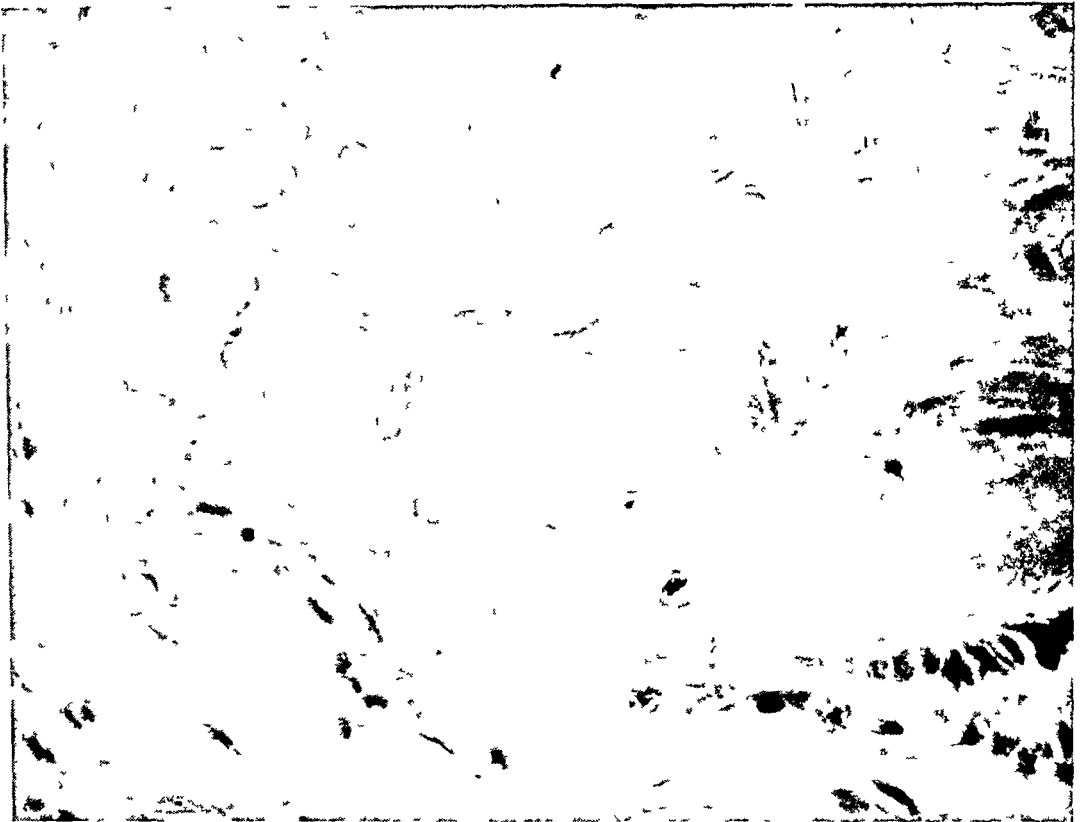


Fig 9—Higher magnification of part of figure 8. Note the continuity at the advancing line of ossification of the collagenous fibers into the bony matrix. Again, note the absence of a rim of osteoblasts at this site of progressing ossification. Hematoxylin and eosin.

#### TUMOR OF HUMAN FEMALE BREAST

A white woman, aged 28, married, was admitted to the Mount Sinai Hospital, Oct 26, 1921. She had 2 children, the last born one year before. A physician had noted a mass about the size of an egg, two and a half years before admission. The tumor grew progressively, with particularly rapid enlargement during the month prior to admission.

The patient was well nourished and developed. Examination of the left breast revealed diffuse enlargement to several times the size of the opposite breast. The overlying skin was smooth, glossy and discolored purple-red. Several

whitish drops of fluid could be expressed through the everted nipple of the involved breast. The tumor was not adherent to the underlying fascia.

*Macroscopic Observations*—The specimen consists of a left breast removed by radical resection. The overlying skin is discolored purple-red but is not retracted. The nipple is everted. Replacing most of the parenchyma is an encapsulated globular cystic tumor, measuring 20 cm in diameter. Its sectioned surface is purple-gray and presents numerous cystic areas filled with gelatinous fluid. The lining of these cysts is papillomatous. The intervening tissue is moderately firm, smooth and purple-gray with scattered whitish areas as well as areas of frank necrosis.

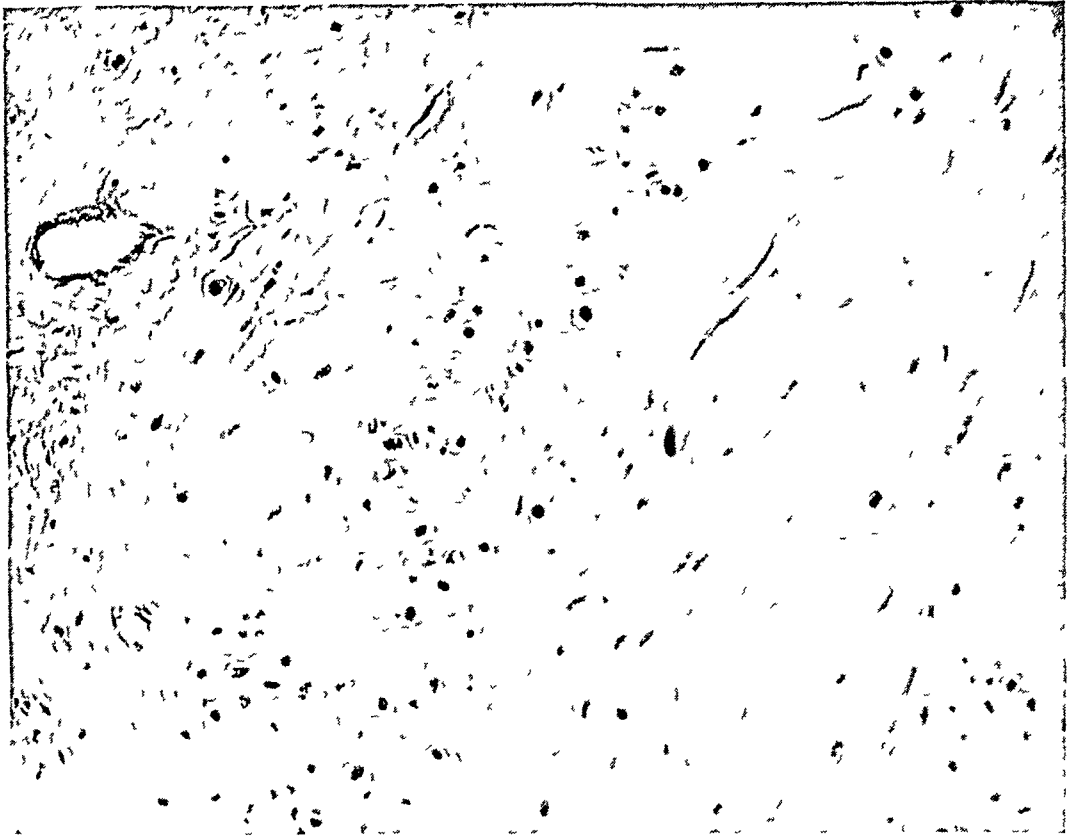


Fig 10—Fibromyxochondrosarcoma of the human breast. This field shows an area of fibrocartilage resulting from metaplasia of *adult, sclerotic* collagenous tissue. Note early formation of lacunae and later transitional stages. Hematoxylin and eosin.

*Microscopic Observations*—Sections reveal the tissue to be composed almost exclusively of collagenous tissue, in which a few single mammary acini are isolated. The fibrous tissue is for the most part markedly cellular. The nuclei show moderate hyperchromatism and irregularity of size and shape. Mitotic figures are not seen. These compact spindle cells frequently merge, on the one hand, with a loose fibrillar, in places myxomatous tissue and, on the other, with a more homogeneous sclerotic tissue, in which the cells are isolated in lacunae so as to present the picture of fibrocartilage. On closer examination, one readily observes the transition of the cellular fibrous tissue to a more sclerotic type and thence

to fibrocartilage without the intervention of the myxomatous tissue. There is *no transition between the myxomatous tissue and the fibrocartilage*. In this process of chondrification the spindle-shaped nuclei with their rim of cytoplasm (fibrocytes) are seen to be isolated from the collagen and come to lie in an oval alcove surrounded by collagenous fibrils. The cell then appears to curl on itself. The alcove becomes circular and forms a typical lacuna, the rim of which stains more intensely than the surrounding fibers, so as to resemble a capsule. At the same time the rim of cytoplasm becomes vacuolated and shrinks, possibly owing to fixation. Some of the lacunae are occupied by two cells, a few of which appear fused, while others are evenly divided by a septum. The glycogen stain is negative. (The specimen is eighteen years old.)

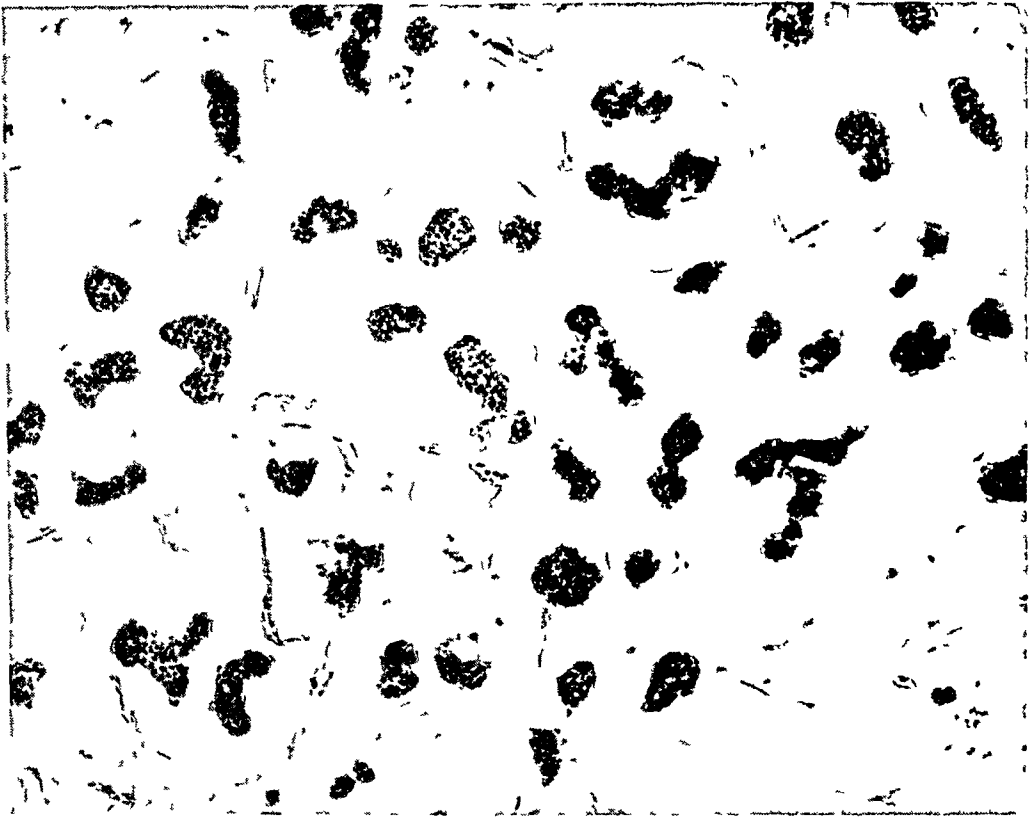


Fig. 11—Colloid carcinoma of the human breast. Contrast the nests of cells in this tumor with the retiform pattern found in the canine tumors. Hematoxylin and eosin.

Several isolated glands show no evidence of proliferation. They are found in all portions of the tumor, including the fibrocartilage.

The lymph nodes are uninvolved.

*Diagnosis*—The tumor is a fibromyxochondrosarcoma.

*Comment*—This tumor represents a sarcoma with transformation of the sclerotic, collagenous portion into fibrocartilage. It is generally stated that fibrocartilage develops directly from immature mesenchymal cells endowed with an inherent tendency in that direction. Such a vitalistic concept precludes the forma-

tion of fibrocartilage *directly* from mature collagenous tissue such as one observes in this tumor. It is true that this sclerotic tissue is part of a tumor, and it might conceivably be argued that the cells may have acquired fibroblastic potencies by dedifferentiation. However, it is the sclerotic, mature, fibromatous portion of the tumor which manifests an easily traced transition to fibrocartilage, rather than the cellular or even myxomatous tissue.

In summary, cartilage was found in 4 canine mammary tumors. In addition, osteoid tissue was present in one of these and bone in another. It appears that the cartilage in 3 of the tumors is epithelial in origin, notwithstanding the orthodox concept of the specificity of germ layers. It is emphasized that this epithelium is ectodermal in origin and that it is not embryonic but typical of the adult type. This epithelial metamorphosis seems to be a mechanistic<sup>21</sup> process, depending on physicochemical phenomena rather than on vitalistic properties with which the cells are inherently endowed. Part of the cartilage in 2 of the tumors, and the osteoid and osseous tissue as well, are considered mesenchymal in origin. The fibrocartilage of the tumor of the human breast appears to arise directly from the mature collagenous tissue rather than from immature mesenchymal cells with cartilaginous propensities. This metamorphosis, too, is regarded as physicochemical in nature and without relationship to specific cellular endowments.

#### THEORIES AS TO THE GENESIS OF CARTILAGE AND BONE IN SO-CALLED MIXED TUMORS OF THE BREAST

The literature on the subject of the genesis of cartilage and bone in so-called mixed tumors of the breast and other organs is highly complicated and controversial. The principal theories that have been advanced—both for human and canine tumors—are based on the following concepts:

##### 1 Embryonic rests<sup>22</sup>

21 The term "mechanistic" as used in this report is contrasted with "vitalistic" and is applied in the sense of a physicochemical explanation of biologic phenomena. See discussion by M. R. Cohen in his "Reason and Nature" (New York, Harcourt Brace & Company, Inc., 1931, pp. 240-292).

22 (a) Cohnheim, J. Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1877, vol. 2, chap. 7. (b) Leser. Beitr. z. path. Anat. u. z. allg. Path. **2**: 379, 1888. (c) Lecene, in discussion on Peyron, M. A. Bull. Assoc. franç. p. l'étude du cancer **13**: 349, 1924. (d) Ribbert, H. Geschwulstlehre, Bonn, F. Cohen, 1904. (e) Borst, M. Echte Geschwülste, in Aschoff, L. Pathologische Anatomie, ed. 8, Jena, Gustav Fischer, 1936, vol. 1, chap. 9. (f) St. Arnold. Virchows Arch. f. path. Anat. **148**: 449, 1897. (g) MacCallum, W. G. A Text-Book of Pathology, ed. 6, Philadelphia, W. B. Saunders Company, 1936, pp. 1193-1194. (h) Wilms<sup>3</sup>. (i) Heuter and Karrenstein<sup>7</sup>. (j) Gomori<sup>8</sup>.



## 2 Local or autochthonous development

(a) Connective tissue metaplasia <sup>23</sup>(b) Epithelial metaplasia <sup>24</sup>

The authors supporting the theory based on this concept account for at least a portion of the cartilage (or "pseudocartilag," as some call it [see page 607]) by epithelial transformation

1 *Embryonic Rests*—The hypothesis that an embryonic rest of cartilage or bone is stimulated to neoplasia after a period of latency is convenient, simple to grasp and, in blanket fashion, skirts the possibilities of intricate cellular transformations. In its favor are said to be (1) the wide diversity of tissues that may occur in a single mixed tumor of the breast and (2) their frequent encapsulation. The source of these skeletogenous rests has been attributed to the ribs or clavicle (Arnold <sup>22f</sup>). This general hypothesis accounting for heterotopic chondral or osseous tissues through dysontogenesis has met with much opposition when applied to a site theoretically more favorable for such occurrences—for example, the parotid region (Krompecher <sup>25</sup>, Masson and Peyron <sup>24a</sup>, Fraser <sup>26</sup>). At least here the more complicated development and the proximity of the cartilage of the branchial arches to the parotid region afford for the adherents to the hypothesis advanced by Cohnheim <sup>22a</sup> a possible basis for the occurrence of misplaced cartilaginous rests. In the breast this possibility based on unappropriated particles of the anlagen of the rib or the clavicle would certainly seem to be very much less. Furthermore, these rests would be obliged to occur much more frequently in the canine than in the human breast in view of the greater incidence of such tumors in the former. If in the process of formation of ribs there is a developmental phenomenon peculiar to dogs which permits of frequent displacement of an anlage, it has not been demonstrated.

Moreover, one can hardly reasonably reconcile such a hypothesis with the finding of mammary acinar tissue directly in the midst of an area of cartilage

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23 (a) Stilling, H. *Deutsche Ztschr f Chir* **15** 247, 1881 (b) Bowlby, A. A. *Tr Path Soc London* **33** 306, 1883 (c) Edelman, H. *Beitr z path Anat u z allg Path* **78** 618, 1927 (d) von Hacker. *Arch f klin Chir* **27** 614, 1882 (e) Chrier, L., and Deval, C. *Bull Soc anat de Paris* **85** 586, 1910 (f) Fry, H. J. B. *J Path & Bact* **36** 529, 1927 (g) Auler, H., and Wernicke. *Ztschr f Krebsforsch* **36** 529, 1927 (h) Durante, in discussion on Peyron, M. A. *Bull Assoc franç p l'étude du cancer* **13** 349, 1924 (i) Cornil, V. *Les tumeurs du sein*, Paris, F. Alcan, 1908 (j) Davidsohn, C. *Ztschr f Gynak* **35** 1357, 1909 (k) Busser, F. *Ann d'anat path* **6** 1247, 1929 (l) Freese, K. *Ztschr f Tiermed* **9** 206, 1905 (m) Imomaki, K. *Gann* **26** 1, 1932 (n) Pitschugin, L. M. *Virchows Arch f path Anat* **280** 136, 1931 (o) Coats, J. *Glasgow M J* **4** 35, 1871 (p) Virchow <sup>1a</sup> (q) Billroth <sup>1b</sup> (r) Sehrt <sup>1c</sup> (s) Kitt <sup>1e</sup> (t) Thunnes <sup>4</sup> (u) Frei <sup>1f</sup>

24 (a) Masson, P., and Peyron, M. A. *Bull Assoc franç p l'étude du cancer* **7** 219, 1914 (b) Masson, P. *ibid* **11** 345, 1922, (c) *Atlas du cancer*, Paris, Association française pour l'étude du cancer, 1924, pts 3 and 4, (d) *Diagnostics de laboratoire II Tumeurs*, Paris, A. Maloine et fils, 1923, p 309 (e) Tudhope, G. R. *J Path & Bact* **48** 499, 1939 (f) Nieberle <sup>2</sup> (g) Ehmann, quoted by Nieberle <sup>2</sup> (h) Ewing <sup>11</sup>

25 Krompecher, E. *Beitr z path Anat u z allg Path* **44** 51 and 87, 1908

26 Fraser, A. *Surg, Gynec & Obst* **27** 19, 1918

or, in other words, in the midst of an embryonic rest (Petit<sup>27</sup>) This is clearly demonstrated in the material presented herewith In addition, some of the cartilaginous foci are not encapsulated but merge with the surrounding tissue The mere encapsulation of the cartilage or the presence of a heterogeneity of tissues need not be evidence of dysontogenesis, as will be explained shortly on the basis of epithelial transformation For these reasons, in addition to those based on histologic studies, the Cohnheim theory, popularized by Wilms<sup>3</sup> in the early part of this century, appears to be giving ground to the hypotheses based on local or autochthonous development of cartilage and bone not only in tumors of the breast but also in those of the parotid region

2 *Local or Autochthonous Development*—(a) *Connective Tissue Metaplasia or Development from Separate Germ Layers* One of the currently advocated hypotheses simplifies the problem somewhat by ascribing the origin of the epithelial and mesenchymal components to the neoplasia of two germ layers, that is to say, the carcinomatous portion is derived from epithelium, and the sarcomatous portion, including the cartilage and bone, is a transformation of mesenchymal tissue A modification of this states that the proliferating epithelium stimulates the neighboring stroma to acquire embryonic potencies with the capacity, accordingly, to develop into a variety of mesenchymal derivatives, including cartilage and bone (Auler and Wernicke<sup>23g</sup>) This is compatible with the hypothesis of Ehrlich and Apolant<sup>28</sup> to the effect that the transformation of the transplantable carcinoma of mice into sarcoma is due to a stimulation of the stroma by the proliferating epithelium Auler and Wernicke<sup>23g</sup> suggested that the necrotic epithelium may furnish the inciting factor

There seems to be no doubt that cartilage or bone may replace connective tissue The histologic mechanism, however, is controvertible and will be further considered on page 621 It is nevertheless a fact that such heterotopic bone formation has been found in almost every mammalian organ, including the blood vessels, heart, lungs, fallopian tubes and uterus, in hematomas, in abdominal wounds and in the stroma of tumors, for example, tumors of the ovary, cecum, rectum and other tissues<sup>29</sup> There is no reason, therefore, to doubt the possibility of a replacement of stromal tissue by cartilage or bone in tumors of the breast A second source of the cartilage and bone is, of course, considered to be the mesenchymal tumor cells themselves

(b) *Epithelial Metaplasia* Contrary to the concept of the specificity of germ layers, it is maintained by some observers that the epithelial cells of certain tumors may give rise to cartilage According to Lang,<sup>30</sup> this thought was advanced

27 Petit, M G Bull Soc anat de Paris **81** 373, 1906

28 Ehrlich, R, and Apolant, H Centralbl f allg Path u path Anat **17** 513, 1906

29 (a) Monckeberg, J G Virchows Arch f path Anat **167** 191, 1902 (b) Bunting, C H J Exper Med **8** 365, 1906 (c) Buerger, L, and Oppenheim, A ibid **10** 354, 1908 (d) Pollack, K Virchows Arch f path Anat **165** 129, 1901 (e) Nicholson, G W J Path & Bact **21** 287, 1917 (f) Poscharissky, J F Beitr z path Anat u z allg Path **38** 135, 1905 (g) Asami, G Am J M Sc **160** 107, 1920 (h) Leriche, R, and Policard, A Les problemes de la physiologie normale et pathologique de l'os, Paris, Masson & Cie, 1926 (i) Chauvin, E, and Rouslacroix, A J d'urol **27** 465, 1929

30 Lang, F J, in Henke, F, and Lubarsch, O Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol 5, pt 2, p 169

as early as 1859 by von Bruns and has since been applied almost exclusively to the so-called mixed tumors of the salivary glands by Marchand,<sup>21</sup> Ehrlich,<sup>16</sup> Krompecher,<sup>25</sup> Masson and Peyron,<sup>24</sup> Fraser and others.<sup>26</sup> It has found support recently in the histologic studies of Nieberle<sup>2</sup> on tumors of the mammary glands of dogs, in Tudhope's<sup>24e</sup> observations on a tumor of the human breast, in Smard's<sup>32</sup> analysis of a tumor of the palm and in the investigations by Foulds<sup>33</sup> of a transplantable tumor of the oviduct of a domestic fowl. This concept enjoys the support also of Ewing.<sup>11</sup>

Three of the tumors described in this paper appear to point unequivocally to the transformation of epithelium first to the transitional stage of a myxomatous tissue and then to cartilage by the process of apparent inspissation and collagenization of a homogeneous *substance fondamentale* between the epithelial cells.

On this basis of epithelial derivation, the presence of carcinoma and of myxoid and chondroid tissue in the same tumor hardly need represent the chaos of a true organoid teratoma but a histologically comprehensible conversion of epithelium with definite transition stages. Nor is it surprising, therefore, that foci of cartilage are sometimes encapsulated, since—according to the interpretation herein advanced—they may arise from nests of glands which were originally surrounded by connective tissue septums. It is maintained that these are additional potent arguments against the theory of enclavement or of embryonic rests as applied to this type of so-called mixed tumor. The well known dermoids or teratomas, for instance, of the ovary, are considered examples of dysontogenesis and have no relation to this discussion.

#### EVIDENCE FROM EMBRYOLOGY OF THE NONSPECIFICITY OF GERM LAYERS

The thought of the mutation of germ layers is naturally somewhat repugnant to the adherents to the orthodox concept of *omnis cellula e cellula eiusdem generis*. They must reconcile, however, the development of cartilage from fetal ectoderm which is described by a number of investigators, including Platt<sup>34</sup> and Landacre and Warren.<sup>35</sup> From the field of experimental embryology, one learns further, through the ingenious investigations on the blastula and gastrula of amphibians, that if cells which were destined to form the epidermis of skin are transplanted to the orbital cavity they may differentiate into nerve, muscle, notochordal, cartilaginous or other cells (Bautzmann<sup>36</sup>, Kusche<sup>37</sup>). This emphasizes the realization that "the strictly preformistic conception of development has been losing ground. We have come to realize that development means the bringing into existence of new characters,

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31 Marchand, F. Verhandl d Gesellsch deutsch Naturforsch u Aerzte **82** (pt 2) 1, 1910.

32 Smard, L. C. Am J Cancer **33** 182, 1938.

33 Foulds, L. A. J Path & Bact **44** 1, 1937.

34 Platt, J. B. Anat Anz **8** 506, 1892.

35 Landacre, F. L., and Warren, J. H. Anat Rec **14** 42, 1918.

36 Bautzmann, H. Naturwissenschaften **17** 818, 1929.

37 Kusche, W. Arch f Entwcklungsmechn d Organ **120** 192, 1929.

not the bringing into appearance of pre-existing latent characters" (Weiss <sup>38</sup>)

Although tempting, one must gird oneself against the direct transfer of such evidence to the tissues—even the neoplastic tissues—of the fully developed organism, as Woglom <sup>13</sup> has indicated. Yet, it does seem to suggest that if a cell is sufficiently immature it possesses a plasticity which allows it to be molded by the multiplicity of physical, chemical and possibly other factors composing the environment. This subjugation of the cell to surrounding forces appears to be the master key to the problem of cellular variability, rather than a vitalistic inherent cellular endowment. The attempt will be made in this report to show that this environmental concept may be applied to the so-called mixed tumors (other than organoid teratomas), in which the cells appear to exhibit a versatility generally conveniently considered to be potentiated by specific endowments but for which a mechanistic explanation appears more tenable and more tangibly applicable to what one observes histologically.

#### "PSEUDOCARTILAGE"

It must be mentioned that many of the authors who describe the same type of transformation of epithelium as observed here are unwilling to concede that the resultant tissue is really cartilage. Accordingly, it is referred to or at least considered as pseudocartilage (for example, Fiy,<sup>23f</sup> Tudhope <sup>24e</sup> and Budd and Breslin <sup>39</sup>). A similar interpretation is applied by many observers to the cartilage in the tumors of the salivary gland (for example, Marchand,<sup>31</sup> Leroux and Leroux-Robert,<sup>40</sup> Bottner,<sup>41</sup> Boyd <sup>42</sup> and Foulds <sup>33</sup> to cartilage in a tumor of the oviduct of a domestic fowl). On the other hand, although their views on the histogenesis may vary, the great majority of investigators nevertheless have expressed the belief that the tissue in both salivary and mammary tumors is true cartilage (for example, MacCallum <sup>22g</sup> and Ewing <sup>11</sup>).

If the concept of the epithelial origin of cartilage has merit, one should hardly rest on the mere histologic resemblance of the epithelial product to cartilage. Rather, one is logically required to assume the burden of matching each of the components of normal cartilage with a component of the "epithelial cartilage" and of tracing the latter from its epithelial progenitor.

38 Weiss, P. *Physiol Rev* **15** 639, 1935

39 Budd, J. W., and Breslin, F. J. *Am J Cancer* **31** 207, 1931

40 Leroux, R., and Leroux-Robert, J. *Bull Assoc franç p l'étude du cancer* **23** 304, 1934

41 Bottner, O. *Beitr z path Anat u z allg Path* **68** 364 1921

42 Boyd, W. *A Text-Book of Pathology*, ed 3, Philadelphia, Lea & Febiger, 1938, p 768

*Chondrocytes* —It has not been shown that the cells in the epithelial cartilage differ in any essential detail of behavior or of morphologic appearance from chondrocytes. In both, distinctly basophilic capsules are present. In places the perilacunar basophilism is widened to a thick cuff, such as is seen commonly in ordinary cartilage. From one to four cells are found within the lacunae, and in an occasional lacuna the cell appears to have dropped out, just as may occur in a section of normal hyaline cartilage. The cells are more or less circular, although some irregularity in shape is present, which is generally ascribed to differences in pressure. The nuclei are for the most part vesicular and contain small nucleoli. Some of the nuclei, however, are pyknotic. Such pyknosis is not uncommon in normal growing cartilage and is attributed usually to poor penetration of the fixing fluid (Shipley<sup>43</sup>). Most strikingly characteristic are the large cytoplasmic vacuoles and the cytoplasmic strands which extend to the lacunar walls as if the remainder of the cell had shrunken. These vacuoles contain abundant globules of glycogen and small fat droplets. The amount of glycogen is pronounced and characteristic, too, of normal hyaline cartilage. In short, the essential features of the cells of normal hyaline cartilage are shared by the cells of the epithelial cartilage herein described.

*Matrix* —It is maintained by others that the matrix of the "pseudo-cartilage" consists merely of a homogeneous mucus surrounding the epithelial cells (for example, Boyd<sup>42</sup> in reference to tumors of the salivary glands). As evidence against such an interpretation, one may mention three specific features common to the matrix of normal cartilage and the matrix of epithelial cartilage, which distinguish the latter from the mucoid intercellular fluid of the nonchondrified portions.

1 The extreme metachromasia with toluidine blue

2 The progressively greater degree of carminophilia with increasing resemblance of the transitional stages to cartilage. It is indeed significant to note that this distribution of glycogen in the epithelial cartilage quite parallels the situation in the developing epiphyseal cartilage. Here, too, the cells of the mature cartilage contain the most glycogen, there being a gradual diminution in the younger, transitional cells (Harris<sup>19</sup>).

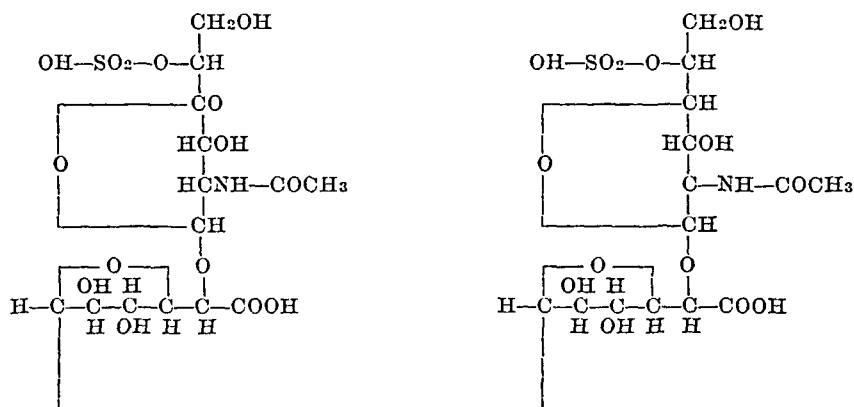
3 The transformation or replacement of the mucoid fluid by collagen.

So much for the general characteristics of the matrix. When one further analyzes the matrix, one finds much to indicate a close relationship between the "mother substance" and the chondral product, particularly from the chemical standpoint. Of course, one cannot state

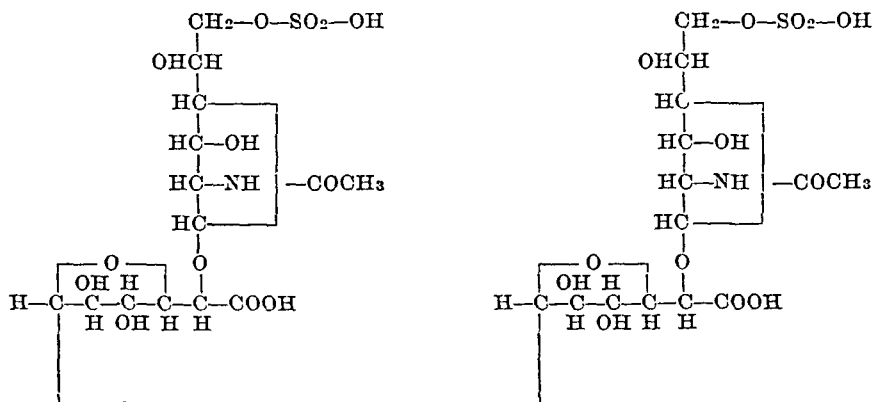
<sup>43</sup> Shipley, P. G. Cartilage and Bone, in Cowdry, E. V. Special Cytology, New York, Paul B. Hoeber, 1928, vol. 2, pp. 703-733.

with certainty the exact chemical nature of the original fluid between the epithelial cells, nor that of the final product of the transformation. However, the former does possess an affinity for carmine and mucicarmine—variable and often slight, to be sure—and so it is generally assumed to be a mucoid fluid with mucicarmine-sulfuric acid as its characteristic constituent. It is relevant, therefore, to indicate by formulas the intimate relationship between this compound and one of the basic organic constituents of the chondroid and osteoid matrix, namely, chondroitin-sulfuric acid.

*Mucicarmine-sulfuric acid*



*Chondroitin-sulfuric acid*



(Levene<sup>44</sup>)

Each compound contains one acetyl group to one molecule of glycuronic acid, sulfuric acid and a sugar. This sugar is chitosamine in mucicarmine-sulfuric acid and chondrosamine in chondroitin-sulfuric acid. The difference lies principally in the fact that the sugars are isomers of each other. The position of the sulfur group is arbitrary and is assigned to indicate a difference in behavior toward hydrolytic agents (Levene<sup>44</sup>).

44 Levene, P. A. Hexosamines. Their Derivatives and Mucins and Mucoids, Monograph 18, Rockefeller Institute for Medical Research, 1922.

This close relationship between mucin and the matrix of cartilage has been previously emphasized by Mathews<sup>45</sup> and recently by Techoueyres<sup>46</sup>. In spite, however, of the fact that the chemical differences are slight, mucoitin has not been converted to chondroitin *in vitro*, and theoretically such a conversion would be most difficult (Sobotka<sup>47</sup>). There appear, therefore, to remain three possibilities for the development of a part of the matrix of cartilage from a mucoid fluid: (1) that the original intercellular fluid and subsequent matrix contain chondroitin rather than mucoitin (chondroitin-sulfuric acid is not confined to cartilage but is present in other locations [Levene<sup>44</sup>] not having the consistency of cartilage, such as tendon, sclera and aorta), (2) that nature, by some unknown method, is able to convert mucoitin into chondroitin, or (3) that the mucoitin retains its chemical composition and becomes part of a matrix with the physical and histologic characteristics of the matrix of cartilage. One must remember, after all, that the exoskeleton of invertebrates is composed of a polysaccharide (polymerized monacetylglucosamine) containing chitosamine and acetic acid as does mucin (Hawk and Berghem<sup>48</sup>). Nevertheless, the exoskeleton is characterized by a hard consistency. It would therefore seem perfectly possible that the mucoid material about the neoplastic cells hardens similarly so as to form a matrix with a consistency resembling cartilage. It is of further interest that some varieties of mucin contain chondroitin-sulfuric acid (Wells<sup>49</sup>). In addition, the conversion of mucoid fluid to collagen is admitted by competent observers<sup>50</sup>.

*Collagenous Component of Cartilage*—The second and by far the most abundant of the basic organic constituents of cartilage is collagen (Maximow and Bloom<sup>51</sup>). One is therefore obliged to account for the conversion of a portion of the mucoid fluid into collagen. It is not within the scope of this report to include the evidence for the various theories of the origin of collagen (see Doljanski and Roulet,<sup>52</sup>

45 Mathews, A. P. *Physiological Chemistry*, ed 5, New York, William Wood & Company, 1931, pp 333-335.

46 Techoueyres, E. *Ann d anat path* **71** 905, 1934.

47 Sobotka, H. Personal communication to the author.

48 Hawk, P. B., and Berghem, O. *Practical Physiological Chemistry*, ed 10, Philadelphia, P. Blakiston's Son & Co., 1931, pp 154 and 207.

49 Wells, H. G. *Chemical Pathology*, ed 2, Philadelphia, W. B. Saunders Company, 1914, p 379.

50 Ehrlich<sup>16</sup> von Ebner<sup>17</sup> Ricker and Schwalb<sup>18</sup> Ewing<sup>11</sup>.

51 Maximow, A. A., and Bloom, W. *A Text-Book of Histology*, ed 3, Philadelphia, W. B. Saunders Company, 1938, chaps 6 and 7.

52 Doljanski, L., and Roulet, F. *Virchows Arch f path Anat* **291** 260, 1933.

Maximow,<sup>53</sup> Arey<sup>54</sup> and Hass<sup>55</sup> for review and bibliographies) It suffices to summarize the principal theories in the following condensed form

1 The intracellular—according to which the fibers originate from (a) the homogeneous peripheral rim of ectoplasm, (b) the mitochondria or cytoplasm proper

2 The intercellular theory—whereby the cells secrete an amorphous sol which is transformed by cellular enzymes into a gel with consequent condensation into fibers of collagen

3 The direct transformation of fibrin (Bartsell<sup>56</sup>) or a *substance fondamentale* (Nageotte<sup>57</sup>) into collagen, a process in which fibroblasts are considered to play an entirely secondary role

The last theory of the direct transformation of an amorphous or fibrinous substance through the action of cellular enzymes or possible mechanical factors has in recent years been receiving increasing attention, principally through the impetus of the observations of Bartsell,<sup>56</sup> Nageotte<sup>57</sup> and Doljanski and Roulet.<sup>52</sup> A direct application of this concept is made to clots, for example, where, it is maintained, the collagen is formed by the gelation of the fibrin of the exudate. Harrison,<sup>58</sup> after a careful study of the development of the amphibian balancer (rodlike props attached to the sides of the heads of larvae), came to the conclusion that collagenous fibers developed in an *acellular* mesenchymal matrix under the enzymatic influence of adjacent *epithelial* cells. As a result of these observations, Ehrlich,<sup>16</sup> von Ebner,<sup>17</sup> Ricker and Schwalb<sup>18</sup> and Ewing<sup>11</sup> are convinced that mucus may be directly transformed into collagen. Finally, it is interesting to note that McKinney,<sup>59</sup> on the basis of studies of tissue cultures, concedes that "it is by no means proved that the fibroblasts are the only cells which are concerned in the formation of collagenous fibers." These details seem, then, to indicate convincingly the possibility of the transformation of mucoid, fibrinous or protein fluids into collagen, which, it is recalled, is one of the principal organic constituents of the matrix both of cartilage and bone. If this transformation becomes a universally recognized fact, a prominent barrier to the acceptance of the mechanistic conception of the conversion of such fluids into a *chondroid* matrix—no matter if the cells concerned are ectodermal or mesenchymal—will have been automatically removed.

53 Maximow, A. A. Bindegewebe und blutbildende Gewebe, in von Mollendorff, W. Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1927, vol. 2, p. 549.

54 Arey, L. B. Physiol. Rev. **16**: 327, 1936.

55 Hass, G. M. Arch. Path. **27**: 583, 1939.

56 Bartsell, G. Am. J. Physiol. **44**: 109, 1917, J. Exper. Med. **21**: 455, 1915, **23**: 739, 1916, Am. Rev. Tuberc. **21**: 593, 1930, Quart. J. Microsc. Sc. **69**: 571, 1924.

57 Nageotte, J. Compt. rend. Soc. de biol. **79**: 833 and 940, 1916, **82**: 277, 1919.



Now, then, the arguments in favor of the tissue within the tumors being "pseudocartilage" are said to be (1) the frequent absence of perichondrium (Leroux and Leroux-Robert<sup>40</sup>), (2) the substitution of epithelial cells for chondroblasts (Tudhope<sup>21e</sup>, Foulds<sup>33</sup>, Leroux and Leroux-Robert<sup>40</sup>), and (3) the persistence of the mucus as matrix instead of a true chondroid matrix (Boyd<sup>42</sup>)

Brief deliberation on the role of the perichondrium in chondrogenesis will show why its presence or its absence is thoroughly irrelevant to the issue concerning "pseudocartilage" The perichondrium is normally a mesenchymal source for additional cartilage Therefore, it is quite as if one were to say, in effect, that the "epithelial cartilage" is not cartilage because it does not arise from mesenchyme Such an argument—based on the very premise which is being disputed, namely, that cartilage may arise only from mesenchymal tissue—obviously can hardly be used as evidence Moreover, to label a tissue "pseudocartilage" simply because its cells are epithelial in origin is to avoid taking into account the facts of the presenting observations As previously stated, these epithelial cells within the cartilage are similar to chondrocytes in all essential details Finally, evidence showing that the mucus does not persist as such but becomes converted into a matrix indistinguishable from chondral matrix has been presented in the preceding paragraphs

In summary, it would therefore seem that under certain conditions strikingly present in the canine mammary tumors (for instance, trauma, inflammation, necrosis, hemorrhage, retiform cellular pattern) epithelial cells of the adult type derived from ectoderm in combination with their interstitial substance may become transformed into a tissue indistinguishable from cartilage The requisites appear to be physicochemical and include a cell—be it fibroblastic, chondroblastic or indeed epithelial—arranged in a more or less reticular pattern or otherwise scattered in a matrix It is entirely conceivable, and consistent with the evidence herein advanced, that this same general mechanism is the basis of normal chondrification as well as ossification This mechanistic interpretation is the central thought of this report and will be further elaborated under "Comment"

#### CHONDRIFICATION IN COLLOID CARCINOMA OF THE HUMAN BREAST

The question that logically arises next is why chondrification does not occur in the colloid (gelatinous or mucous) carcinoma of the human breast, inasmuch as an apparently chondrifiable milieu is present One may wonder whether or not the mucoid fluid of the canine breast differs chemically from that of the human breast, since mucins in other parts of the body are known to differ from each other in chemical composition

58 Harrison, R G J Exper Zool **41** 349, 1925

59 McKinney, R L Arch f exper Zellforsch **9** 14, 1929

and properties (Levene<sup>44</sup>) There are no known data on this point, and it must therefore remain moot for the present However, a significant detail concerning this gelatinous fluid is the fact that it does occasionally manifest an affinity for calcium (Shore<sup>60</sup>) This affinity is demonstrable in one of the cases included in this report and tends to suggest that the reason for the failure to chondrify lies possibly not with the mucin but with the viability and arrangement of the cells

*Polarity*—The question of the arrangement of the epithelial cells in these and other colloid carcinomas is of great interest The phenomenon is apparently dependent on cellular polarity Masson,<sup>24b</sup> in his investigations of mixed tumors of the parotid gland, laid much stress on this feature of cellular function The polarity of the cell apparently determines whether a group of cells will assume an acinar, nestlike fascicular or reticular pattern For instance, if the cell possesses an interstitial or diffuse polarity, its secreting surface is diffuse instead of at one side The obvious result of such a diffuse secreting surface is that the fluid accumulates on all sides of neighboring cells, which are consequently forced apart and assume first a spongy, and then a myxomatous, pattern One immediately conceives of a direct application of this to the problem of the canine tumors and the human tumors of the colloid variety It will be recalled that the colloid carcinomas were composed not of reticulated epithelial cells but of solid clumps which showed practically no evidence of intercellular accumulation of fluid (fig 11) The fluid appeared rather to surround the nests of epithelium An application of Masson's scheme seems to indicate that there is a difference in polarity between the clumps of epithelial cells of the colloid carcinoma of the human breast as opposed to the cells in the canine mammary tumor At any rate, the difference in arrangement of the cells is regarded of major importance in the failure of chondrification of the colloid not only in the human breast but also in the colloid or mucous carcinomas of other organs, for example, the gastroenteric tract and the ovary The cells within the alveoli or cysts of these tumors are generally cast-off, degenerating cells or at least cells which are destined soon to degenerate As a matter of fact, Geschickter<sup>61</sup> expressed the belief that the mucus formed in the colloid carcinoma of the human breast is related actually to interference with the blood supply

It is maintained that by the very nature of their generally more or less retiform pattern of growth, as opposed to the solid or alveolar pattern of carcinoma, the sarcoma—in quite the same way as proliferating fibroblasts—lends itself much more easily to chondrification as well as ossification because of the ease with which such cells are separated by the transuded or secreted fluid or by a collagenous tissue

60 Shore, B R Am J Cancer **15** 221, 1931

61 Geschickter, C F Ann Surg **108** 321, 1938

undergoing mucoid change. It must be borne in mind after all, that soft tissue sarcomas do form bone not altogether infrequently. It is only when the epithelial cells—even epithelial cells of the adult type—simulate this arrangement, as in the canine mammary tumors, the mixed tumors of the salivary glands and other regions, and possibly in tumors of the type described by Woglom,<sup>13</sup> that the stage for chondrification is set. This pattern, independent of inherent cellular potencies of a specific nature, appears to me to be the key to the phenomenon of “epithelial chondrification.” An illustration of this general principle is afforded by the transplantable mammary carcinoma of mice studied by Woglom.<sup>13</sup> In the second generation the cells became spindle shaped. Sarcomatous transformation was thought to have occurred, and, indeed, cartilage was found in 3 of the 4 tumors of this generation. The very presence of cartilage was considered strong evidence that the tumor had become sarcomatous. However, one must reckon with the fact that cells of epithelial tumors (for example, adamantinoma, thyroid carcinoma, basal cell carcinoma) may acquire a spindle shape (Ewing<sup>62</sup>) and that even in cultures of epithelial cells it may be impossible to distinguish some of the cells from fibroblasts.<sup>63</sup> As a matter of fact, this same transplantable carcinoma of mice which was regarded as having undergone sarcomatous change was considered by Ewing<sup>62</sup> to be actually a spindle cell form of carcinoma. Slye, Holmes and Wells<sup>12</sup> described a somewhat similar spontaneous tumor of a mouse. One does not mean to capitalize on these differences in interpretation of an exceedingly difficult problem; they are mentioned merely because they at least tend to bear out the possibility that the proliferating cell taking part in the process of chondrification may, on occasions, be ectodermal as well as mesenchymal.

#### REASONS FOR DISPARITY IN INCIDENCE OF CARTILAGE AND BONE IN CANINE AND HUMAN MAMMARY TUMORS

Closely related to the questions of histogenesis is the second phase of the problem, which concerns itself with the reasons for the disparity in incidence of cartilage and bone-containing tumors of the mammary glands of dogs and man. Peyron<sup>64</sup> maintained that the increased incidence in dogs is directly attributable to the abundance of myoepithelial cells in this species as contrasted with man. This cannot be confirmed. Glendinning<sup>18</sup> suggested that there is an “apparent interchangeability of mesoblastic structures” of the dog because they are less highly specialized than in man. If Glendinning meant to say that the stromal tissue of the canine mammary gland is not as mature or possesses a greater lability than the corresponding tissue of the human breast, he must bear the burden of a proof which has not been demonstrated.

<sup>62</sup> Ewing, J. J. Cancer Research **1** 71, 1916. Ewing<sup>11</sup>

<sup>63</sup> Nordmann, M. Arch. f. exper. Zellforsch. **9** 54, 1929.

<sup>64</sup> Peyron, M. A. Bull. Assoc. franç. p. l'étude du cancer **13** 349, 1924.

The following factors suggest themselves as having a possible bearing on the question

1 The acknowledged tendency toward sarcomatous change in the mammary glands of dogs as contrasted with man

2 The greater liability of the canine mammary glands to trauma, inflammation, hemorrhage and necrosis

3 A possible species difference in bone-forming propensity

1 *Sarcomatous Tendency*—The well recognized tendency for sarcomatous change to take place in the mammary gland of the dog as contrasted with man is considered of importance in the production of cartilage and bone not because of any inherent specific potency in that direction but for the following two reasons

(a) As stated, from the mechanistic point of view the sarcomatous structure is suited for the formation of cartilage and bone by virtue of its retiform pattern and the consequent ease of separation of cells when the fluid is secreted or transuded

(b) Sarcomas are exceedingly prone to necrosis, hemorrhage and inflammation—factors which are held to be of significance in the general phenomenon of the formation of cartilage and bone. This is to be discussed in more detail in the following paragraphs

2 *Trauma, Necrosis, Hemorrhage and Inflammation*—The role of trauma in the chondrification and ossification of tumors of the canine breast merits consideration. It is a fact that these tumors occur predominantly in the hindmost breast (Niebeile<sup>2</sup>). This is the largest of the glands, and during lactation it secretes the most milk and becomes the most pendulous. The subcutaneous tissue is loosest in this region which permits the gland of the active animal to swing freely and thereby to subject itself to repeated trauma and strain

The possible role of trauma in the proliferation and tumefaction of cartilage and bone has been appreciated for a long time. It is of course thoroughly appreciated how difficult it may be to evaluate an etiologic agent of such a character. This and related points were justifiably emphasized by Ewing<sup>65</sup>. Behan,<sup>66</sup> who recently reviewed the subject comprehensively, included an extensive bibliography of observers who favor the thesis that trauma has a distinct bearing on the initiation and further development of tumors of the skeleton. On the other hand, a not inconsiderable number were cited who discount any such relationship. Virchow<sup>1a</sup> strongly emphasized the factor of trauma in the causation of enchondroma and pointed out that the sites of these tumors were the sites of liability to trauma. Modern statistics confirm the relationship between the skeletogenous tumor and the specific history of definite

65 Ewing, J. Arch Path **19** 690, 1935

66 Behan, R. J. The Relationship of Trauma to New Growths, Baltimore, Williams & Wilkins Company, 1939

trauma (Geschickter and Copeland<sup>67</sup>, Kolodny<sup>68</sup>) The relation of mechanical factors, such as trauma, stress and strain, to chondrification and ossification has been repeatedly demonstrated also in vitro (for instance, Glucksmann<sup>69</sup>)

Even if trauma were to be regarded as an exciting factor in the proliferation of cartilage and bone, it would be appreciated that the objection might be raised that skeletogenous tumors may not be strictly comparable to soft tissue tumors containing cartilage and bone The difference lies really in the assumption that in the former there are present tissues which are directly chondrogenic or osteogenic, whereas in the latter such tissues are lacking However, this assumed difference is hardly the fact inasmuch as cells of soft tissue sarcomas, and even epithelium under certain circumstances, may become chondrogenic or osteogenic (Mallory<sup>70</sup>) This potentiality, it need hardly be mentioned, is not nearly as marked as it is in the periosteum or perichondrium, and by the same token the incidence of bony and cartilaginous tumors is not nearly as great in the soft parts as in the skeleton The point is that this difference in incidence does not preclude a general factor or combination of factors, such as trauma, stress, strain hemorrhage necrosis and inflammation, as part of the setup which initiates chondrification and ossification That a metaplasia of connective tissue into cartilage may take place under the influence of mechanical factors can hardly be doubted, according to Maximow and Bloom<sup>51</sup>

It must be stated clearly that the issue of present concern is not related to the question of the relation of trauma to the initiation of carcinoma or sarcoma but simply to the matter of cartilage and bone production Moreover, it is desired emphatically to avoid the implication that trauma per se—that is, the mere application of force—is responsible for chondrification or ossification The important features of trauma in this regard are its sequelae, namely, interference with circulation, hemorrhage, necrosis, edema and inflammation It is not difficult to understand that inflammation with edema, necrosis, ulceration and hemorrhage within the tissues occurs frequently in sites so exposed and subjected to repeated trauma as are the tumors of the hindmost canine mammary glands Contrasted with the canine tumor, the human mammary carcinoma is, as a rule, relatively small in proportion to the size of the breast and is cushioned by much fat The canine tumor, on the other hand, is generally exposed to the surface or covered merely by a thin capsule and skin (fig 7) so that the impact of trauma to the region is borne by the neoplasm Inflammation, with hemorrhage

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67 Geschickter, C F, and Copeland, M M Tumors of Bone, New York American Journal of Cancer, 1931

68 Kolodny, A Surg, Gynec & Obst, 1927, supp 1, p 1

69 Glucksmann, A Anat Rec 73 33, 1939

70 Mallory, T B Am J Path (supp) 9 765, 1933

and necrosis, was present in the 4 canine growths and in the partially chondrified human tumor included here. The large size and rapidly growing sarcomatous nature of the latter neoplasm predisposed it toward hemorrhage, necrosis and inflammation. These factors, it is maintained, are of major importance in the tendency toward calcification, chondrification and ossification of the mammary gland of the dog in the same way as they are considered to play the predominant role in the calcification and ossification of a variety of other organs subjected to these influences.<sup>71</sup> In this connection, one should like to make the point that the parotid region suffers a great deal more constantly repeated trauma by the simple act of mastication than is generally appreciated. One can evaluate the degree of this trauma roughly by palpation of the masseter muscles with the teeth clenched as in chewing. Indeed, about a quarter of a century ago, Fraser<sup>26</sup> postulated that trauma was an important factor in the origin of mixed tumors of the salivary glands.

3 *Experimental Ossification Under the Influence of Epithelium*—From the field of experimental surgery, one learns the interesting fact that when fascia is transplanted to an artificial defect in the urinary bladder of a dog bone forms within the fascia (Neuhof<sup>72</sup>). Moreover, transplantation of a strip of epithelium from the urinary bladder of a dog to the rectus fascia constantly causes bone to be formed directly adjacent to the border of the transplanted epithelium (Huggins,<sup>73</sup> Abbott, Goodwin and Stephenson<sup>74</sup>). The same experiment in rabbits produced bone in only 1 of 6 animals.<sup>73</sup> The administration of large doses of viosterol and parathyroid to such experimental rabbits failed to stimulate ossification at the sites of the transplanted epithelium. Connective tissue transplants to the urinary bladders of rabbits produced no bone in contrast to the results in dogs (Phemister<sup>75</sup>). Ossification was observed also in guinea pigs and rats by Huggins, McCarroll and Blocksom,<sup>76</sup> but to a decidedly lesser extent by Abbott, Goodwin and Stephenson<sup>74</sup> in their guinea pigs and cats. On the other hand, Gruber<sup>71b</sup> found it difficult to produce traumatic myositis ossificans in dogs as contrasted with rabbits.

It is further worth noting that the epithelial transplants to the fasciae of the dogs formed cysts containing usually about 2 to 4 cc of fluid,

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71 (a) Harvey, W. H. J. M. Research **12** 25, 1907. (b) Gruber, G. B. Ueber Histologie und Pathogenese der circumskripten Muskelverknöcherung, Jena, Gustav Fischer, 1913. (c) Sacerdotti, C., and Frattin, G. Virchows Arch f. path. Anat. **168** 431, 1902. (d) Virchow<sup>1a</sup>. (e) Bunting<sup>29b</sup>. (f) Leriche and Policard<sup>29b</sup>.

72 Neuhof, H. Surg., Gynec. & Obst. **24** 383, 1917.

73 Huggins, C. Arch. Surg. **22** 377, 1931.

74 Abbott, A. C., Goodwin, A. M., and Stephenson, E. J. Urol. **40** 294, 1938.

75 Phemister, D. B. Ann. Surg. **78** 239, 1923.

76 Huggins, C. B., McCarroll, H. R., and Blocksom, B. H. Arch. Surg. **32** 915, 1936.

which was examined for calcium, phosphorus and hydrogen ion concentration<sup>73</sup> The values for calcium and phosphorus in the cysts were strikingly higher than those for these elements in the blood, and the  $p_H$  was distinctly lower On the other hand, the values of phosphorus and of  $p_H$  for the corresponding 3 rabbits quite matched those of the blood (Calcium values were not given) In other words, these data indicate a fundamental mensurable difference in the ossifying ability of at least the dog and rabbit and imply a significant role of the epithelium in the production of bone under these conditions As to what the corresponding results in the human subject would be, there are unfortunately no data If such data were available, one might then, it is not unlikely, possess an additional clue to the problem of the disparity in the incidence of cartilage and bone in tumors of the mammary glands of dogs and of man on the basis of species differences Chemical examination of the mucoid material of both human and canine breasts not only for glycoproteins but for calcium, phosphorus, phosphatase and  $p_H$  might furnish additional significant data

Of course, if there is in fact a specific tendency toward ossification in dogs, one might reasonably expect evidence thereof in tumors other than those of the mammary glands And to be sure, according to Ruddock,<sup>77</sup> ossification in malignant growths of the thyroid occurs most frequently in dogs However, in view of the high incidence of malignant changes of the thyroid in these animals (Slye, Holmes and Wells<sup>78</sup>) this observation might best be subjected to a statistical analysis

#### COMMENT

1 The observation of the development of cartilage from *adult* epithelium of the mammary gland of the dog is the pivot about which most of this discussion turns This observation would constitute a mere academic minutia if one were to concern oneself merely with the mammary gland However, to state in effect that cartilage, a mesenchymal derivative, may be formed directly from an adult ectodermal derivative, or that the chondrocyte may be replaced by an epithelial cell, is to challenge the orthodox concept of the specificity of germ layers—a concept which forms the basis of present day teaching of many phases of biology Adherents to this concept might explain this observation in one of two ways

(a) That it represents a vitalistic monstrosity, with anaplasia of the epithelium to immature, multipotent cells, followed by differentiation into cartilage

(b) That the tissue is not true cartilage

<sup>77</sup> Ruddock, H B, and Willis, R A Am J Cancer **33** 205, 1938

<sup>78</sup> Slye, M, Holmes, H F, and Wells, H G J Cancer Research **10** 175, 1926

In the text of this report, many reasons have been advanced to indicate the inadequacy of the explanations offered by this conveniently flexible concept of the vitalists, who must necessarily meet problems of this sort merely by turning the clock backward or forward or, if it suits the occasion, in both directions. One is not justified in assuming that the mere anaplasia of the cells of an adult organism, even though accompanied by the properties of rapidity of growth and invasiveness, *ever* carries with it the other potentialities of fetal cells. More decisive, however, is the fact that the epithelium concerned in this chondrification is, histologically at least, of the adult type—being portions of obviously mature mammary acinar epithelium. In answer to the second, more serious objection it is stated that this epithelial cartilage has been matched component for component with normal cartilage as far as known morphologic, biochemical (pancreatic digestion) and tinctorial criteria permit, and found indistinguishable therefrom.

It is maintained that the development of this cartilage from adult epithelium is the result of environmental influences, and, to be sure, the observations of the transitional stages comply neatly with a mechanistic explanation. This interpretation of the chondrification of the epithelium is compatible with the "newer" embryology, which attempts to eliminate the preformistic concept of cellular endowments and attributes the characteristics of a cell to the dictates of its environment (Weiss<sup>38</sup>). This is vividly illustrated by the development of a blastulous cell known ordinarily to form epidermis of skin, which when transplanted may develop into muscle, notochordal cartilaginous or other cells. In other words, it is not meant to imply that if the individual cells of the epithelial cartilage were transplanted to tissue cultures they would necessarily form cartilage, for the reason that the environmental factors would be changed and might therefore not be suitable.

2 The second ramification of this basic phenomenon concerns the matrix. The intercellular fluid, originally of a mucoid nature, becomes transformed into or replaced by a collagenized matrix indistinguishable from a chondral matrix. Is this a true transformation of mucin directly into collagen—a phenomenon acknowledged by several prominent observers (Ewing,<sup>11</sup> Ehrlich,<sup>16</sup> von Ebner,<sup>17</sup> Ricker and Schwalb<sup>18</sup>)—or has the mucin been replaced by collagen derived from the surrounding connective tissue, possibly with the participation of the mucin? As stated, the chemical relationship of mucoitin to chondroitin is close, just as the mucin and the final chondral matrix appear to be morphologically in these tumors. However, an accurate positive statement as to which of these two possibilities is correct is obviously impossible, although it appears not unlikely that both the intercellular mucoid fluid and the preexisting collagen take part in the formation of the final matrix.



3 A more immediate question concerns the interpretation of the heterogeneity of the tissues in these tumors or, in other words, the term "mixed tumor." In one tumor (dog 166) the bone and part of the cartilage were formed by transformation of the stromal tissue. Inasmuch as such ossification and chondrification, even though extensive, as in this case are really simply heterotopic stromal formations, this type of tumor would more properly be referred to as a carcinoma or a sarcoma with stromal ossification or chondrification, rather than as a mixed tumor. In those neoplasms in which the tumor cells themselves take part in the formation of bone or cartilage, the qualifying terms "myxo-," "chondrio-," "osteoid-" or "osteo-" should be used to modify "carcinoma" or "sarcoma." For example, the diagnosis myxochondroadenocarcinoma should imply that the myxomatous and cartilaginous cells are epithelial in origin (as, for example, in dog 322). Similarly, the term "osteosarcoma" should be applied to corresponding soft tissue tumors. The diagnosis *osteogenic sarcoma*, as Kolodny<sup>68</sup> suggested, ought then to be restricted to tumors arising directly from the skeleton.

4 In one tumor (dog 166) small foci of cartilage were observed forming nearby large bony trabeculae. The process seemed to be not an ossification of cartilage but an independent formation of each from adjacent portions of the same tissue, that is, the stroma. This is confirmatory evidence for the more or less generally accepted belief that the cells taking part in the formation of cartilage may be of the same type as those participating in ossification. More direct evidence is the observation of ossification of cells of the perichondrium, on the one hand, and of chondrification of periosteal cells, on the other. This may be observed *in vitro* (Glucksmann<sup>69</sup>).

5 The development of fibrocartilage from the mature, sclerotic collagenous tissue of the fibromyxochondrosarcoma of the human breast raises an additional fundamental question. As stated, one observes the transformation of the collagenous tissue into fibrocartilage quite as if the cartilaginous formation occurred after the adult collagenous tissue had been formed. Generally fibrocartilage is assumed to develop from immature mesenchymal cells with a particular potency for chondrification. The precise environmental factors responsible for this aberration are unknown at present, although it is probable that they, too, are stress, strain, inflammation and necrosis. Such a phenomenon, as well as the production of cartilage from epithelium, adds to the accumulating evidence against the specificity of chondroblasts.

6 Closely related to the question of the direct transformation of collagenous tissue to fibrocartilage is that of heterotopic ossification. The significance of the very familiar picture observed in foci of stromal ossification ought to be reconsidered in the light of certain histologic observations. One notes a transition between the ossified matrix and

the adjacent mature collagen of the stroma (figs 8 and 9) One can actually observe the collagenous fibers from the surrounding stroma continuing on into the bony trabecula The process seems to consist first of a homogenization of the collagen, due perhaps to necrosis, hemorrhage, inflammation and other processes This homogenized collagen possesses an affinity for calcium The cells of the original collagen—the fibrocytes—are imprisoned by the matrix and in this situation are called osteocytes The result of this transformation is a bony trabecula, in which transformation the role of the cells appears to be of entirely secondary importance

These same figures illustrate a second significant point At *A* (fig 8) one observes the line of advancing ossification At this site, however, one notes the practical absence of cells that might be called osteoblasts This is especially striking in contrast with the other surfaces of the same trabecula If osteoblasts were responsible for this progressive ossification, one would logically expect to see them active at the line of advancing ossification It would seem, therefore, that the new bony matrix in this type of ossification is derived not from osteoblasts but from a direct transformation of nonspecific collagen with nonspecific fibrocytes

This observation and interpretation are not new Similar examples are found classically in ossified tuberculous complexes Weidenreich<sup>79</sup> mentioned several investigators other than himself who had observed the same type of picture and had applied a similar interpretation Direct opposition to the vitalistic concept of the specificity of the osteoblast has been voiced in clearcut terms by Leriche and Policard,<sup>29h</sup> Grieg<sup>80</sup> and Murray<sup>81</sup> T B Mallory<sup>70</sup> concluded from his observations of ossified soft tissue sarcomas that any fibroblast may take over the function of the osteoblast, a view stated in 1914 by F B Mallory<sup>82</sup>

However, this mechanistic concept of heterotopic bone formation continues to meet with the resistance of the adherents to the traditional concept of intramembranous ossification as given in textbooks For example, Ham<sup>83</sup> stated in Cowdry's "Special Cytology" that "there is no evidence to show that metaplastic bone develops by a metamorphosis

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79 Weidenreich, F Das Knochengewebe, in von Mollendorff, W Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol 2, pt 2, p 435

80 Grieg, D M Clinical Observations on the Surgical Pathology of Bone, London, Oliver & Boyd, 1931, chap 1

81 Murray, C R J Dent Research **11** 837, 1931

82 Mallory, F B The Principles of Pathologic Histology, Philadelphia, W B Saunders Company, 1914, pp 275-277

83 Ham, A W, in Cowdry, E V Special Cytology, ed 2, New York, Paul B Hoeber, Inc, 1932, vol 2, p 990

of adult tissues" Rhode,<sup>84</sup> in an extensive article, emphatically denied the possibility of ossification by direct transformation of connective tissue and insisted that bone is invariably formed from osteoblasts or undifferentiated, "unused remaining mesenchymal cells" These osteoblasts are assumed to secrete or to become peripherally transformed into matrix (Shipley<sup>43</sup>, Maximow and Bloom<sup>51</sup>) Any increase in size of the trabecula is considered to be due to the activity of the osteoblasts

However, there is yet a second familiar important morphologic observation, in addition to that of the direct transformation of collagen to bony matrix, which is explained inadequately by the standard concept, namely, the regular alinement of osteoblasts against the surfaces of a trabecula (fig 8) This commonplace finding is generally regarded as a reflection of a behavior characteristically peculiar to osteoblasts and in a measure indicative of their specificity Maximow and Bloom<sup>51</sup> stated that the increase in size of the trabecula is due to the secretory activity of the osteoblasts They appear not to have accounted for the alinement other than to state that "the osteoblasts gradually move to the exterior" Does it not seem likely, on the one hand, if individual cells were secreting their varying amounts of matrix, that the trabecular surface would tend to be distinctly irregular and the cells irregularly alined? On the other hand, does it not seem more reasonable that the interstitial substance is originally of a fluid nature, derived perhaps from tissue fluids, lymph or plasma, and that the alinement is a compression phenomenon simulating in a sense the alinement of crumbs by a bread crumber or of debris on the beach by the incoming waves?

Regarding the origin of the interstitial substance from plasma, one is reminded of the hyalinoid material of the *Trummerfeld* zone and *Gerustmark* in scurvy, which is generally considered to be formed in part by the osteoid secretion of osteoblasts (for instance, Wolbach and Howe<sup>85</sup>) but which Aschoff and Koch<sup>86</sup> regarded fibrinous in nature The presence of blood pigment in the *Gerustmark*<sup>86</sup> and the hemorrhagic tendency in this disease fortify the suggestion of the intravascular origin of this material This is of utmost significance, because this hyalinoid material becomes transformed into the matrix of osteoid tissue

It is relevant here to refer again to the fundamental observations of Battsell, Harrison, Nageotte and Doljanski and Roulet on the development of collagen from plasma or serous fluids with no apparent or only an indirect influence of cells These observations furnish the basis for the formulation of a theory of intramembranous ossification which seems to be more directly in accord with the morphologic facts and which

84 Rhode, C Surg, Gynec & Obst **41** 740, 1925

85 Wolbach, S B, and Howe, P R Arch Path **1** 1, 1926

86 Aschoff, L, and Koch, W Skorbut Eine pathologisch-anatomische Studie, Jena, Gustav Fischer, 1919

does not circumvent the observations of heterotopic ossification by the use of vitalistic hypotheses based on ubiquitous mesenchymal cells of a specific nature

The formation of collagen directly by the gelation of the colloidal sols of plasma serum or protein tissue fluids is considered to supply the foundation for the development of the matrix of osteoid tissue, of which collagen is the principal organic component. This fluid or, later, semisolid matrix aligns the cells in the vicinity in the characteristic osteoblastic rim by virtue of its lateral pressure against them. If too few cells are present, naturally no such rim is formed, if the bone is developing adjacent to or in the midst of young, cellular, easily compressible granulation tissue, obviously the *likelihood of such an alignment is increased to the degree of the cellularity and compressibility*. The addition of further increments of interstitial substance to the newly developing trabecula appears to be responsible for the lamellations. With the increase in matrix, many of the osteoblasts are forced aside in a regular line by the wave of new material, others are imprisoned as osteocytes. This substance becomes further elaborated into osteoid matrix, and finally calcification occurs.

The evidence is not convincing by any means that participation even in the biochemical processes concerned with calcification is a specific property of osteoblasts. It must be recalled, for example, that in the kidneys of certain animals the phosphatase activity is fully 50 per cent of that of an equal weight of epiphyseal cartilage (Robinson<sup>87</sup>). An interesting experiment illustrating the immediate unimportance of living cells in the process of calcification of cartilage under certain conditions is furnished by Wells and Benson<sup>88</sup>. They heated pieces of epiphyseal, rib and tracheal cartilage to a temperature judged sufficient to kill the cells and placed the pieces in the peritoneal cavity for a varying number of weeks. The calcification of the epiphyseal cartilage was found to be strikingly greater than that of either the rib or the tracheal cartilage. In other words, that cartilage which would have normally ossified in situ became calcified in the peritoneal cavity, whereas the other cartilages—costal and tracheal—which normally do not ossify in situ, showed relatively little calcification. To repeat, this phenomenon occurred in the absence of living cells or of "vital action"<sup>88</sup> within the cartilages.

The phenomenon of heterotopic ossification dovetails easily into the concept outlined, which stresses, first, the extracellular development of both collagen and osteoid matrix and, second, the close relationship of collagen to osteoid matrix and of both to a common interstitial substance. The collagen of the involved connective tissue is changed from its neighboring collagen by a variety of possible factors, such as trauma,

87 Robinson, R. Biochem J **17** 286, 1923

88 Wells, H. G., and Benson, R. L. J. M. Research **12** 15, 1907

stress, strain, necrosis and inflammation, with resulting homogenization and acquisition of an affinity for calcium

#### SUMMARY

Four so-called mixed tumors from mammary glands of dogs and one from a human breast are described. These tumors contain myxomatous tissue, cartilage, osteoid tissue and bone. The tumor of the human breast is a fibromyxochondrosarcoma. Three of the canine tumors are carcinomas, the fourth is a sarcoma.

Contrary to the generally held concepts of the specificity of germ layers, the cartilage in three of the canine neoplasms appears to be *derived directly from adult epithelium*. The transition stages of the conversion of the epithelium into cartilage are easily followed and consist of (1) loosening of the acinar epithelium, (2) isolation of the epithelial cells in a matrix so as to simulate myxomatous tissue, (3) collagenization of the mucoid matrix and (4) homogenization and lacunar formation.

This "epithelial cartilage" is considered to be true cartilage as judged by known morphologic, biochemical (pancreatic digestion) and tinctorial criteria.

The development of fibrocartilage from mature, sclerotic collagenous tissue rather than directly from immature mesenchymal cells has been observed and its significance indicated.

A mechanistic theory of intramembranous ossification is outlined which accounts for both normal and heterotopic ossification without the use of the traditional vitalistic hypothesis of specific osteoblasts and ubiquitous undifferentiated mesenchymal cells.

Reasons favoring the nonspecificity of chondroblasts and osteoblasts are presented, with emphasis on the importance of environmental factors, in agreement with the "newer," mechanistic embryology.

The term "mixed tumor" as applied to this type of neoplasm is misleading and, if used at all, should be reserved for tumors composed of dysontogenetic tissues, such as organoid teratomas. "Myxo-," "chondro-," "osteoid-" or "osteo-" should be added to "carcinoma" or "sarcoma" to designate the type of tumor described here when the myxo, chondro, osteoid or osseous tissue is a direct modification of the tumor tissue. The presence of heterotopic stromal chondrification or ossification should of course not be indicated by qualifying adjectives.

Considerations similar to the foregoing are obviously applicable to other tumors of this type—for example, the so-called mixed tumors of the salivary glands.

Reasons for the strikingly high incidence of cartilage and bone in tumors of the mammary glands of dogs as contrasted with man are given. These are based on the liability of the former to trauma, with resulting necrosis, hemorrhage, inflammation and edema, and on the retiform pattern of the canine acinar epithelium.

# EVALUATION OF THE APPARENTLY INCREASED INCIDENCE OF PRIMARY CARCINOMA OF THE LUNG

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Prior to 1900 primary carcinoma of the lung was believed to be exceedingly rare, and when a tumor was found in the lung it was almost always considered to be metastatic. Now carcinoma of the lung is thought to be quite common, ranking with carcinoma of the stomach, breast and uterus in frequency. Although the evidence suggests strongly that there have been both a relative and an actual increase in primary carcinoma of the lung, physicians should investigate carefully other possibilities before accepting this as a proved fact.

In any attempt to conform with changing ideas of tumor origin, it is natural that certain pendulum-like tendencies should develop. It is my purpose in this paper to suggest that such may be the case in the diagnosis of primary carcinoma of the lung.

It would be interesting in this connection to study the incidence of endothelioma of the pleura over a number of years. Unfortunately information on the incidence of this tumor is not readily available. Certainly the diagnosis was relatively common a few years ago, while now most of the cases are thought to be instances of primary carcinoma of the lung. The old "oat cell" tumor of the mediastinum, so commonly mentioned in English writings, is now thought by most pathologists to be primary in the lungs rather than in the lymph nodes. And much more recently the Pancoast tumor of the superior pulmonary sulcus appears to be slipping into the classification of primary carcinoma of the lung.

Most publications on the subject of primary carcinoma of the lung stress the multiplicity of clinical and histologic types. This in itself is enough to give one pause. Furthermore, the gross criteria are inexact. In most instances the tumor is thought to be primary in the bronchi, and involvement of the bronchial mucosa is a helpful diagnostic point at autopsy. This is not an adequate criterion, however, as will be shown later, in a good many instances a tumor primary elsewhere metastasizes to the bronchi. Some writers state that primary carcinoma of the lung

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may be manifest as bilateral multiple discrete tumor masses. This would seem to let down the bars to all sorts of tumors, primary elsewhere and metastatic in the lungs. I have recently seen a case of this sort, with multiple discrete tumors of about equal size in both lungs, diagnosed as one of primary carcinoma of the lung, although the autopsy had been restricted. The operator was a surgeon who rarely did an autopsy and he stated that the pancreas was indurated and the liver contained nodules. No sections were obtained from the pancreas.

Even in the most experienced hands a primary tumor can be easily overlooked, especially if located in the nasopharynx. Furthermore, many cases of carcinoma show, at the time of necropsy, large masses throughout the body, any one of which could have been primary. It would appear that these could be easily differentiated on the basis of the cellular picture, but in many instances that is not the case. It is the poorly differentiated adenocarcinoma that is apt to give most difficulty in this regard and in such cases the tissue of origin may never be determined certainly. The direction and character of the metastases are frequently of help in arriving at a conclusion.

A case in point will illustrate the difficulties that may be encountered.

#### CASE 1

An emaciated 70 year old white man was admitted to the Gallinger Municipal Hospital in March 1939, with a history of difficulty in swallowing and breathing. There was a brief note on the hospital record that a tumor had been removed from the "throat" at another hospital in March 1937. There was a mass about 3 cm in diameter just above the middle of the right clavicle. Physical examination revealed nothing else remarkable. The difficulty in swallowing became more severe, and a gastrostomy was done under local anesthesia. The man died ten days later, apparently of pneumonia.

Autopsy disclosed an abscess 10 cm in diameter in the anterior portion of the lower lobe of the left lung, near the apex of the heart. A bronchus 8 mm in diameter, leading into that portion of the lung which contained the abscess, was almost occluded by a soft intrabronchial tumor growth (fig 1). The whole growth in this area measured 1.5 cm in diameter. Several smaller nodules of tumor tissue were located near the bronchial growth, and flat umbilicated nodules were noted on the pleural surfaces of both lungs. Two lymph nodes in the hilus were greatly enlarged and contained tumor tissue. The mass in the right side of the neck consisted of neoplasm in lymph nodes there, intimately surrounding the right vagus and accessory nerves and infiltrating their sheaths. The jugular vein in the same area was completely obliterated by tumor growth. The larynx, esophagus and trachea showed no evidence of ulceration, tumefaction, scarring or other abnormality.

This case at the time of autopsy was thought to be one of primary bronchogenic carcinoma with metastasis to the mediastinal and cervical lymph nodes. The involvement of the vagus and accessory nerves was thought to have caused the dysphagia, which was the most conspicuous

clinical symptom in the case. The stenosing bronchial tumor, associated with a mediastinal metastasis and with a pulmonary abscess, appeared typical of primary carcinoma of the bronchus. Histologically, the tumor was composed of rather undifferentiated cells, obviously of squamous type, this is, of course, a common structure for primary carcinoma of the bronchus. On reviewing the case, however, I was struck by the fact

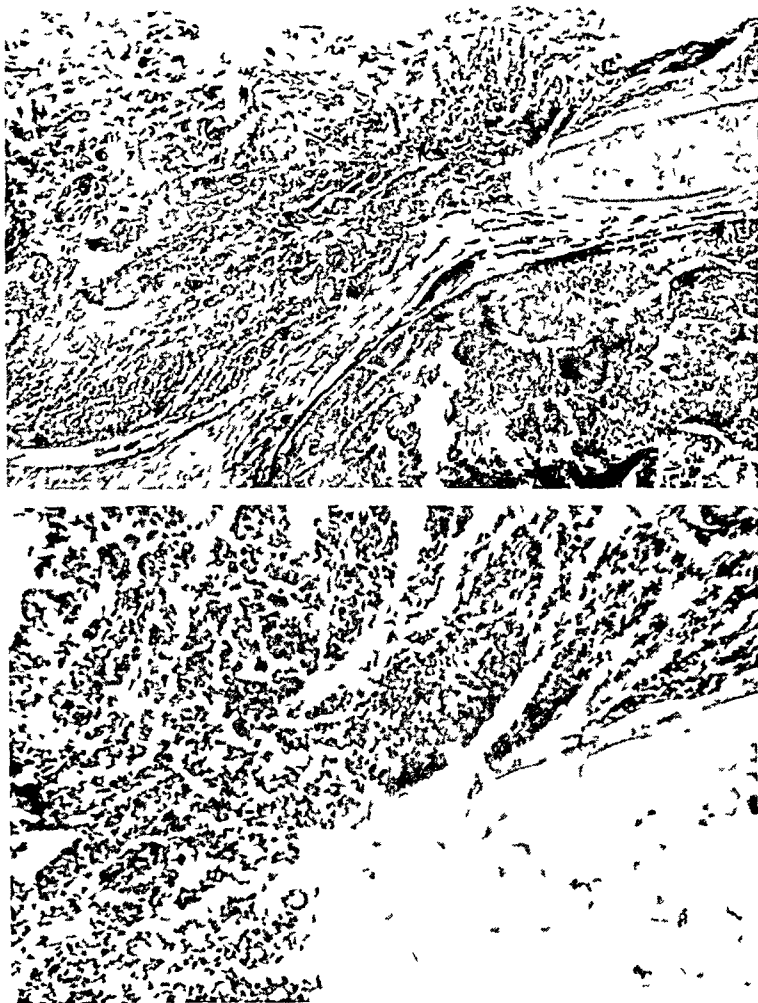


Fig 1 (case 1) —Epidermoid carcinoma of a bronchus, metastatic from a carcinoma of the larynx. At autopsy there were a stenosing bronchial lesion, an abscess of the lung and a mediastinal metastasis. This growth was thought at first to be primary in the bronchus. Above a longitudinal section of the bronchus,  $\times 20$ . Below a part of the same,  $\times 100$ .

that there was a large mass in the *right* side of the neck, that the growth in the lung was on the *left* and that there was no definite chain of lymph nodes connecting the two. As a routine check-up, microscopic section and the record of the original tumor were examined. The biopsy speci-



men had been taken from the esophagus at about the level of the larynx and was diagnosed as epidermoid carcinoma, its cells were indistinguishable from those in the section of bronchus. Subsequently roentgen ray treatment had been given, and the mucosal lesion of the esophagus had been observed to disappear.

This was undoubtedly an instance of metastasis to the bronchus and lung from a carcinoma primary in the esophagus. The primary tumor had healed completely as a result of roentgen ray therapy, leaving tumor tissue viable only in the metastases.

Involvement of the bronchial mucosa is not a satisfactory criterion for stating that a tumor is primary in the bronchus. Metastasis to the bronchial mucosa is fairly common in a wide variety of tumors. Any tumor involving the hilar lymph nodes may extend along the lymphatics directly into the mucosa, and tumor cells may reach the mucosa as a lymphatic extension from metastatic tumors brought to the lung by the blood stream.

In the other 2 cases to be cited the diagnosis was more obvious.

#### CASE 2

A 47 year old Negro woman underwent a radical mastectomy at the Gallinger Municipal Hospital in June 1938 for carcinoma of the breast. In February 1939 she was readmitted because of dyspnea, loss of weight and of appetite, and swelling of the left arm and hand. Autopsy showed a bronchus 1 cm in diameter in the lower lobe of the right lung to be almost completely occluded by tumor tissue (fig 2). There were widespread metastases in both lungs and in the hilar lymph nodes. There were tumor deposits in the axillas, and the growth there had infiltrated a tributary of the axillary vein, whence tumor cell emboli could be carried to the lung. In the lung the tumor probably grew along the lymphatic routes and reached the hilar lymph nodes, and thence reached the bronchial mucosa, or extension could have been directly from the lymphatics of the alveoli into the lymphatic channels of the bronchial mucosa.

#### CASE 3

A 16 year old Negro boy was admitted to the Gallinger Municipal Hospital in April 1937 because of cough and loss of weight. There was rather marked enlargement of the cervical lymph nodes. Biopsy of one of these nodes established the diagnosis of Hodgkin's disease. On a second admission, in November 1937, mediastinal masses were demonstrated by roentgenogram. Subsequently masses developed in the abdomen, and the final admission, in May 1938, was because of enlargement of the abdomen and weakness. Roentgen ray treatment was given intermittently over the neck and abdomen between the last two admissions. Death occurred in July 1938. Autopsy disclosed, in addition to Hodgkin's disease of the liver and spleen and of the lymph nodes generally, a flat nodular growth in the right main bronchus and almost complete occlusion of the bronchus to the middle lobe of the right lung by Hodgkin's tissue (fig 3). There was also invasion of the branches of the right pulmonary artery and vein, as well as of the lung parenchyma about the hilus. The tumor growth in the bronchi, great vessels

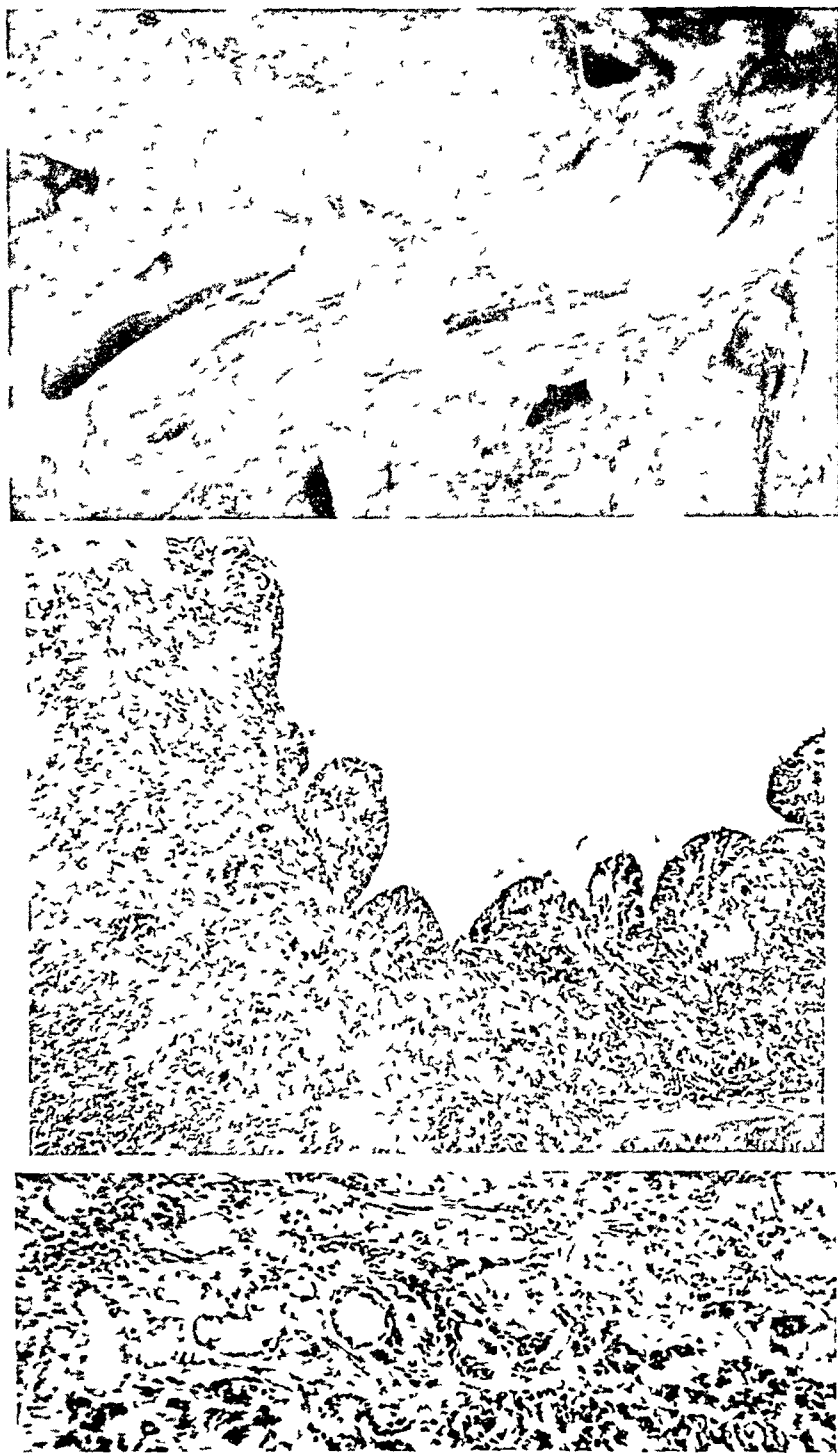


Fig 2 (case 2) —Adenocarcinoma of a bronchus, metastatic from a tumor of a breast. Above gross photograph, about actual size, showing flat nodular neoplastic growth filling the lumen and infiltrating the wall of the bronchus, simulating primary bronchogenic carcinoma. Middle cross section of the bronchus, showing tumor cells in the mucosa and submucosa,  $\times 20$ . Below a part of the same, showing tumor cells infiltrating among the bronchial mucous glands,  $\times 100$ .

and lung parenchyma appeared to be a result of direct extension along the lymphatics from the hilar lymph nodes

The last 2 cases are cited not because there was any difficulty in differentiating the lesions from those of primary bronchogenic car-

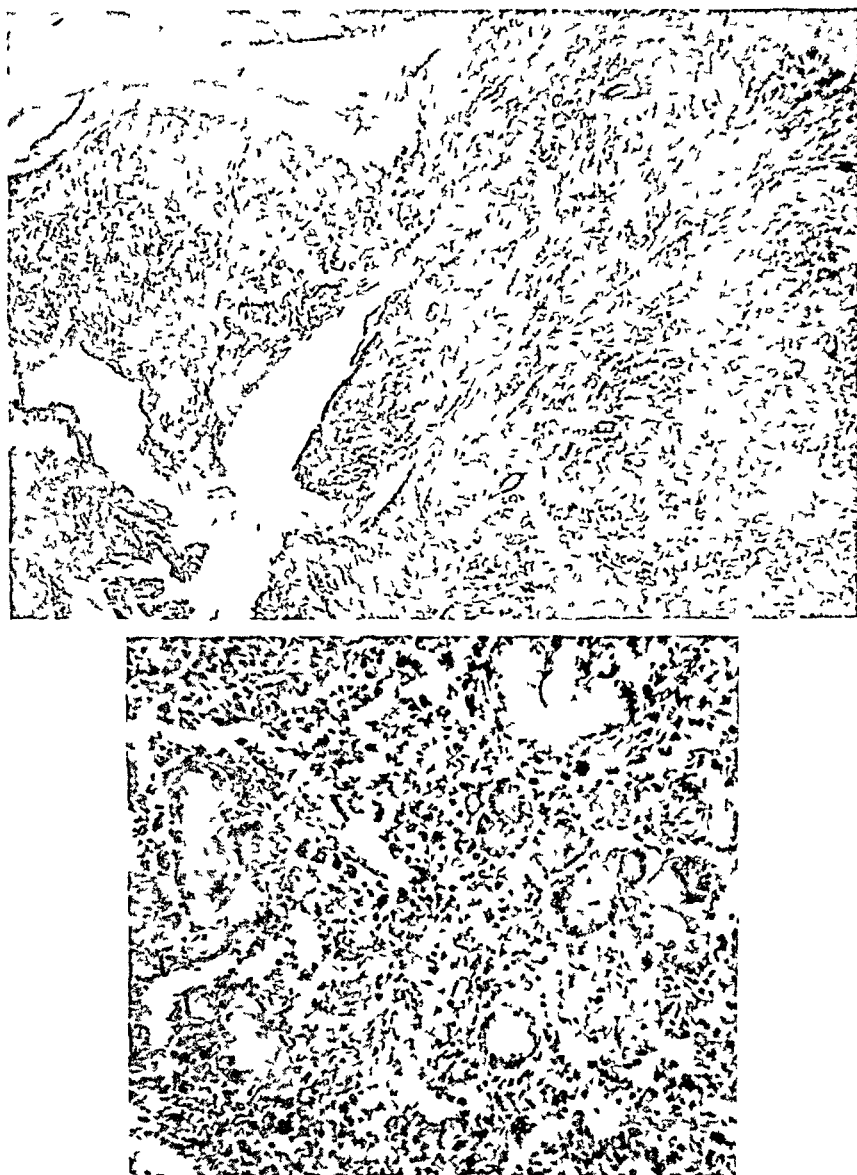


Fig 3 (case 3) —Hodgkin's disease of a bronchus to illustrate involvement of the bronchial mucosa by growths not primary there Above cross section of the bronchus, the lumen of which is almost completely filled by Hodgkin's tissue,  $\times 20$  Below a part of the same, showing Hodgkin's tissue among the mucous glands of the bronchus,  $\times 100$

cinoma grossly but because there was extensive involvement of the bronchial mucosa by metastatic tumor Microscopically, however, the differentiation would be more difficult The solid sheets of rather

anaplastic cells shown in figure 2, infiltrating among the glands of the bronchial mucosa, could not be identified as from the breast by studying that section alone

Since the relative and the actual incidence of any type of neoplasm are usually determined from autopsy statistics, the final responsibility for determining the frequency of a certain type of tumor rests on the pathologist. Generally this practice results in a high degree of accuracy, but it has its pitfalls, as most pathologists will readily admit. Autopsies do not reveal the answer to a clinical question as a cut and dried mathematical derivation, since the judgment of the pathologist and sometimes his whims and fancies come into play. Witness the Krukenberg tumor, long classified as a primary tumor of the ovaries, primary carcinoma of the liver, which was thought to be extremely rare at one time, liposarcoma and neurosarcoma, which have emerged from the older round cell and spindle cell sarcomas thought to be of fascial origin.

It is obvious that there is no ready solution to the problem presented. The most logical approach for the pathologist is to submit every case of carcinoma of the lung encountered at autopsy to an unusually thorough investigation. If tumor masses are found outside the pulmonary system, the case should be studied very carefully to determine which is the primary lesion. It seems doubtful to me that a tumor should be considered primary in the lung when discrete nodules of equal size are distributed more or less uniformly throughout both lungs.

#### SUMMARY

One should hesitate in concluding that primary carcinoma of the lung is increasing in frequency. It is suggested that statistics in this field are probably misleading, for the following reasons:

- 1 Prior to 1900 primary carcinoma of the lung was probably diagnosed less frequently than it actually occurred, even after autopsy, because of the belief then current that the tumor was exceedingly rare. Most of the tumors of this type occurring at that time were considered metastatic, probably erroneously.

- 2 Possibly the diagnosis of cancer of the lung is made more frequently now than is justifiable. Involvement of the bronchial mucosa, generally considered to be one of the criteria for the diagnosis, is not reliable, since metastases to the bronchial mucosa are relatively common. The success of surgical operation and of the application of radium and the roentgen ray in eliminating accessible primary growths, while the secondary tumors remain viable and growing, places an obligation on both the clinician and the pathologist to evaluate carefully the findings in every case.

3 The virtual abandonment of the diagnoses "endothelioma of the pleura," "oat cell tumor of the mediastinum" and "tumor of the superior pulmonary sulcus" and the placing of all the tumors formerly designated by these terms in the classification of primary carcinoma of the lung, has enlarged the group considerably. These tumors appear to me to be correctly classified as primary carcinoma of the lung, but the change has swollen the statistics rather than increased the incidence of the disease.

Undoubtedly primary carcinoma of the lung is quite common, but the alarming apparent increase in the incidence of the tumor in reported statistics cannot justifiably be accepted as an actual increase until due allowances are made for the pendulum to come to rest.

# EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

## I PATHOLOGIC CHANGES IN THE ORGANS OF RATS PRODUCED BY CHRONIC NITRITE POISONING

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An urgent need exists for additional information concerning the etiologic factors and the causative mechanism of the noninfectious degenerative and sclerosing lesions of the arterial walls and their degenerative parenchymatous sequelae in the organs involved, especially the heart, kidney and brain. Among the various endogenous factors which have been incriminated in the production of arteriosclerosis, some are of general chemical or hormonal nature (elevation of the cholesterol and calcium levels of the blood, as in diabetes and hyperparathyroidism, respectively, hyperadrenalism, renal ischemia, causing hypertension), while others are of local type (locally restricted special mechanical strain, vibrations, as at bifurcations). The experimental production of arteriosclerosis in animals by prolonged administration of cholesterol or epinephrine and by restriction of the blood flow in the renal arteries, respectively, has lent support to the contention that these factors play a causative role.

On the premise that the fundamental and general direct causative mechanism active in the development of atheromatous, arterionecrotic and arteriosclerotic lesions is represented by an impairment of the nutrition of the inner portions of the vascular walls (which are not supplied with blood by the vasa vasorum) and that this condition may be brought about by a great variety of factors acting in different ways, the postulate is advanced that hypotensive agents administered over a sufficiently long period and in adequate amounts should exert the same effect on the vascular walls as have been observed in connection with hypertensive agents. To test this hypothesis, rats were placed under the prolonged influence of a hypotension-producing chemical, erythrol tetranitrate.

This communication embraces a report on the vascular and organic changes produced by such a procedure.

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Warner Institute for Therapeutic Research

## CONSIDERATIONS IN SELECTION OF EXPERIMENTAL MATERIAL

In selecting erythrol tetranitrate from the large group of nitrites and nitrates producing nitrite effects (sodium nitrite, amyl nitrite, nitroglycerin, mannitol pentanitrate, ethylene glycol dinitrate, erythrol tetranitrate and others) special consideration was given to the fact that this chemical causes not only an appreciable but a prolonged lowering of the blood pressure, in contrast to other nitrites, which have a more transitory effect in this respect. By employing this chemical, it was thought, animals could be kept for the greater part of the day under the influence of nitrite action.

Rats were chosen as test animals since they spontaneously acquire arteriosclerotic lesions in vessels of various organs (heart, lungs, kidney, testis) with old age, thus displaying a vascular reactivity similar to that observed in man, with whom, moreover, they have in common an exposure to nutritive influences of the same type, both being omnivores. The selection of rats was further influenced by the fact that recent pathologic and histologic investigations (Hueper<sup>1</sup>, Wilens and Sproul<sup>2</sup>, Hummel and Barnes<sup>3</sup>) have provided detailed knowledge concerning the incidence as to age, the character and the extent of the arteriosclerotic lesions occurring in rats. These animals have been used successfully, moreover, for the production of experimental hypertension by the method of renal ischemia developed by Goldblatt<sup>4</sup>.

## EXPERIMENTAL PROCEDURE

Three experiments were conducted. In the first, 30 male rats, approximately 2 months old and weighing between 52 and 110 Gm (average, 77 Gm), were placed on a stock diet adequate in all respects. Increasing amounts of erythrol tetranitrate were added to the food. The chemical was obtained in tablets (tetranitrol, Merck) containing 0.015 Gm each. As erythrol tetranitrate is sparingly soluble in water (1:20,000) but readily soluble in alcohol, a suitable number of tablets were dissolved in 95 per cent alcohol, leaving an insoluble mineral residue. This solution was shaken vigorously, after which approximately 50 cc was removed and added to the food in each cage, containing 6 rats. The initial daily dose per rat was 0.00035 Gm of erythrol tetranitrate. The dose was increased every week by 25 per cent of the amount given during the preceding week. At the end of the experimental period of thirty-two weeks the daily amount of erythrol tetranitrate administered with the food per rat had reached 0.064 Gm, an increase of approximately twenty times.

Inasmuch as the conditions prevailing in the experiment with chronic oral administration neither insured exact control of the dose consumed by the individual animal nor permitted determination of the quantity of nitrite actually resorbed into the organism, a series of 12 female rats was given subcutaneous

1 Hueper, W. C. *Arch Path* **20** 708, 1935, **27** 466, 1939.

2 Wilens, S. L., and Sproul, E. E. *Am J Path* **14** 177, 1938.

3 Hummel, K. P., and Barnes, L. L. *Am J Path* **14** 121, 1938.

4 Goldblatt, H. *Am J Path* **15** 619, 1939, *Bull New York Acad Med* **14** 523, 1938.

injections of a saline suspension of erythrol tetranitrate over a period of one hundred and forty days (twenty weeks) on five days of the week (a total of eighty injections). The dose administered daily was 0.014 Gm during the first week and 0.028 Gm thereafter for the remainder of the experiment. The injections were interrupted on four occasions for the duration of one week, to permit healing of ulcerative lesions formed as the result of the subcutaneous introduction of the suspended solid material. The rats in this experiment were approximately 3 months old at the start and weighed from 118 to 158 Gm (average, 124 Gm).

The third experiment was devised to permit study of the organic and especially the cardiovascular effect of massive doses of an inorganic nitrite, sodium nitrite, for purposes of comparison. Six rats were used, weighing between 160 and 236 Gm at the start of the experiment. They were placed on a meat diet, to which 0.4 Gm of sodium nitrite per rat was added daily. After two weeks the daily dose was decreased to 0.20 Gm, as the rats did not consume their entire ration. After another week it was doubled again and three weeks later was increased to 0.5 Gm. The rats were maintained on this diet until the last survivors of the series were killed, after being in the experiment for eighteen weeks.

#### BIOLOGIC OBSERVATIONS

Sixteen of the 30 rats placed on chronic oral administration of erythrol tetranitrate survived for thirty-two weeks. They were then killed by bleeding from the jugular vein. During the experimental period a constant rise in weight was recorded, ranging at the time of death (when they were approximately 9 months old) from 200 to 376 Gm (average, 262 Gm). Nine rats of this series died or were killed during the first three months of the experiment because of an apparent inflammatory infectious condition of the middle ear, while 5 additional rats died during a later part.

Of the rats given subcutaneous injections, 10 were alive at the end of the experiment. The animals gained only slowly, their weights remaining stationary during the first two months. There was a subsequent gain which brought the weights at the end of the experiment to between 175 and 212 Gm (average, 199 Gm). In the course of the experiment several rats became aggressive and vicious engaging in frequent and violent fights, especially after the injection of the nitrate.

The rats of the sodium nitrite series were listless and exhibited a cyanotic color of skin, which was especially prominent in the tails, ears and feet. All rats except one showed a more or less marked loss of weight. Two rats of this series died during the first two weeks of the experiment, and 2 additional ones were killed after being in the experiment for four weeks.

#### OBSERVATIONS AT AUTOPSY

*Macroscopic Changes*—The rats belonging to the two erythrol tetranitrate series had relatively few macroscopically demonstrable organic lesions. In the oral series several rats which died in the course of the experiment had hyperemic meninges, small flabby brown livers, enlarged, greenish brown kidneys and small soft testes. In 7 other rats there were yellowish to greenish yellow abscesses involving the occipital region of the brain, adjacent to the region of the middle ear. The rats belonging to the sodium nitrite series showed, on the other hand, meningeal vessels filled to the point of congestion with brownish blood, flattened cerebral gyri, collapsed brownish lungs, a soft brown liver, dark red kidneys, small testes and coffee ground-colored mucosal erosions in the stomach, which contained similarly colored material. The intestine was congested and in places was filled with hemorrhagic material.



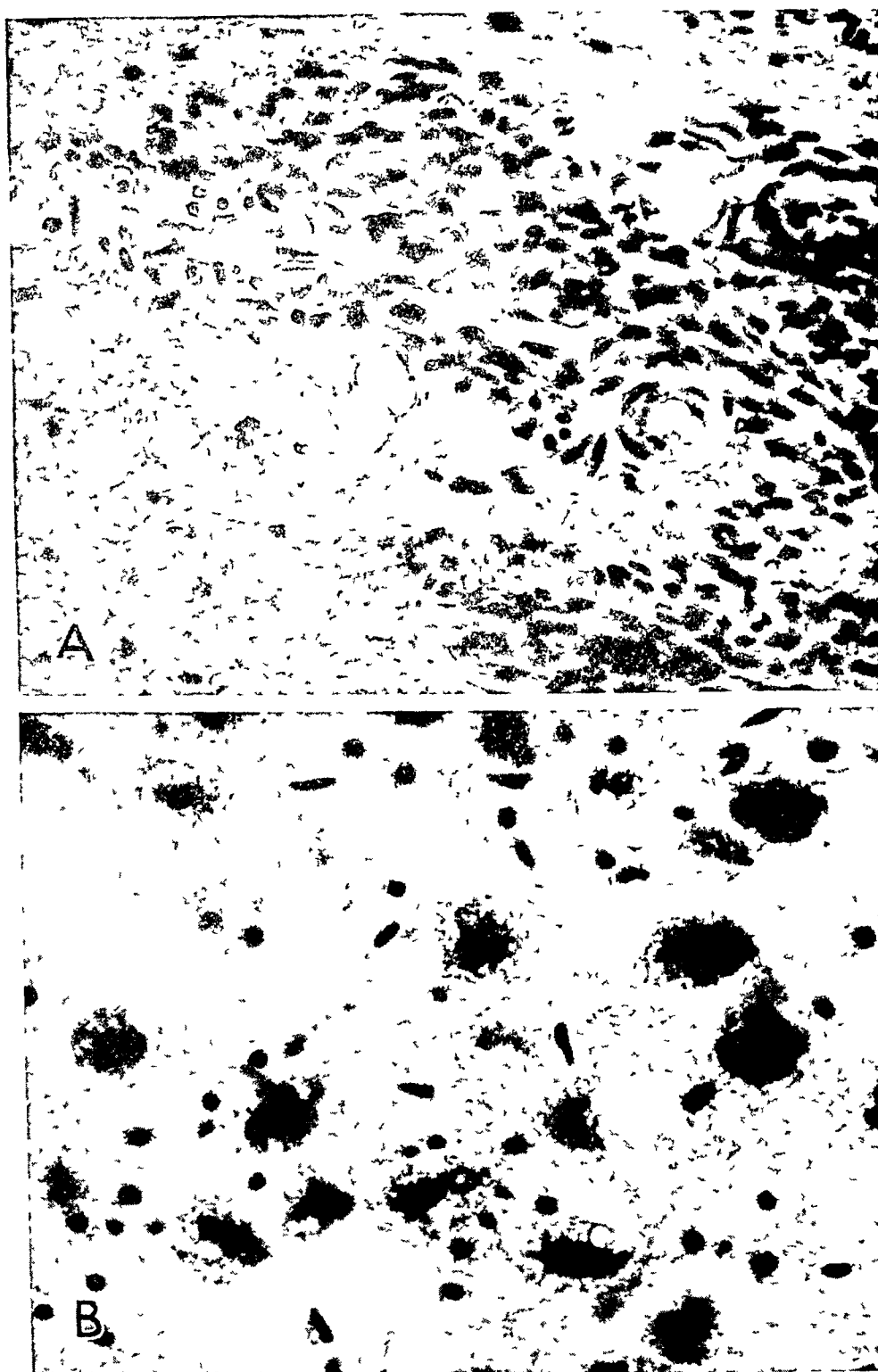


Fig 1—*A*, perivascular fibroblastic proliferation and lymphocytic infiltration with marked edema of the surrounding nerve tissue,  $\times 450$  *B*, extensive vacuolar degeneration of ganglion cells in a center of the brain stem,  $\times 450$

*Microscopic Changes*—Inasmuch as the histologic changes observed in the organs of rats of all three series were essentially identical, varying mainly in degree and extent, it appears appropriate to present them together, especially as they supplement each other in certain respects. Sections were prepared from the following organs: brain, heart, lung, aorta, thyroid, submaxillary gland, stomach, intestine, pancreas, liver, spleen, lymph nodes, adrenal, kidney, bladder, prostate, seminal vesicles, epididymis, testis and bone marrow of the sternum and femur. The sections were stained with hematoxylin-eosin, sections of the brain were stained in addition with toluidine blue, sections of the kidney and heart were prepared with Van Gieson's connective tissue stain, sections of the spleen and kidney were stained by the prussian blue method for iron-containing pigments.

(a) *Brain* If the brains of 8 rats containing abscesses in the occipital region are excluded from further consideration, as these lesions could be ascribed to infections of the middle ear, the following changes were observed in the majority of animals examined. The meningeal membranes were often edematous and cellular and contained engorged vessels. Intrameningeal hemorrhages were seen occasionally. Focal or more or less diffuse lymphocytic infiltrations of the meninges were rather common. The intracerebral vessels were often congested and surrounded either by perivascular edema or small hemorrhages. Perivascular glia cell accumulations and fibroblastic proliferations were found not infrequently (fig 1*A*). Endothelial proliferation of the precapillary and capillary vessels was marked in some rats, causing obliteration of the lumens. In a considerable proportion of the rats the nervous substance contained either local or more or less diffuse glial cell infiltrations, involving mainly the region of the brain stem and of the cerebellum. There were small focal necroses with ameboid gliosis in several rats. Edema of the brain, sometimes associated with marked vacuolation (Swiss cheese-like picture), was observed in several animals. These lesions were often complicated by acute and chronic degenerative changes of the ganglion cells, especially those composing the centers of the brain stem (fig 1*B*).

(b) *Heart* While the myocardium and coronary vessels were normal in some of the rats, many of the animals exhibited degenerative myocardial as well as coronary vascular lesions. In a few animals these changes consisted of marked vascular congestion and multiple small hemorrhagic or plasmatic extravasations. In addition to myocardial edema, there was usually an increase of interstitial histiocytic cells. Hyaline and vacuolar myocardial degenerations were found in a moderate number of rats, whereas focal fibroblastic scars existed in several (fig 2*A*). The lesions most frequently found in the hearts of these animals, however, involved the walls of the coronary myocardial vessels. The media was swollen and completely or partially hyalinized. The nuclei were much enlarged and irregularly round and were sometimes surrounded by a vacuolar zone. In addition to a frequent reduction in the number of nuclei, there was often a localized complete loss of these elements. Histiocytic cells were occasionally seen within the thickened, hyalinized media. The lumens of some of these pathologic vessels were narrowed or showed a compressed oblong shape (figs 2*A* and 3*A*). Perivascular edema was seen in a moderate number of animals.

(c) *Lung* An appreciable number of rats had numerous polypous as well as plaque-like calcifications in the subendothelial spaces as well as in the media of large and small branches of the pulmonary artery. The base of the calcified spurs was usually located within a break of the media. In some small arterioles the calcified projections were so large that they occluded the greater part of the lumen. The nuclei of the smooth muscle cells located in the stumps of the media were irregularly arranged in small clusters, and fibroblastic cells were interspersed with the muscle cells. The pulmonary parenchyma was normal in general.

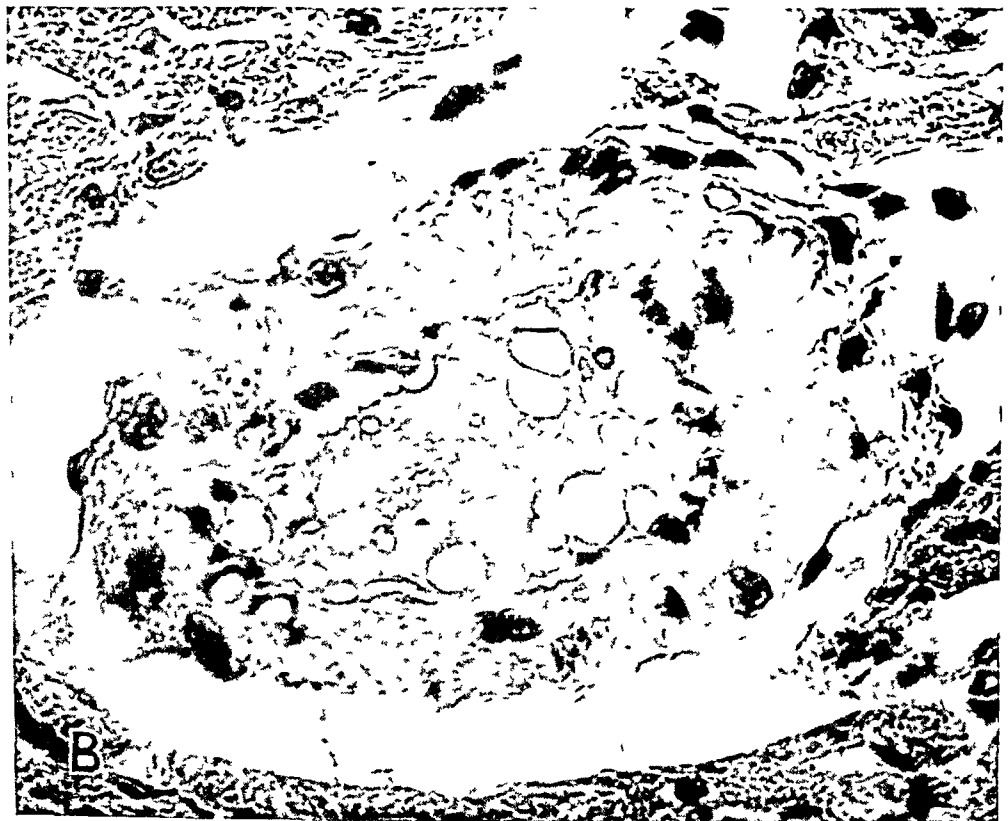
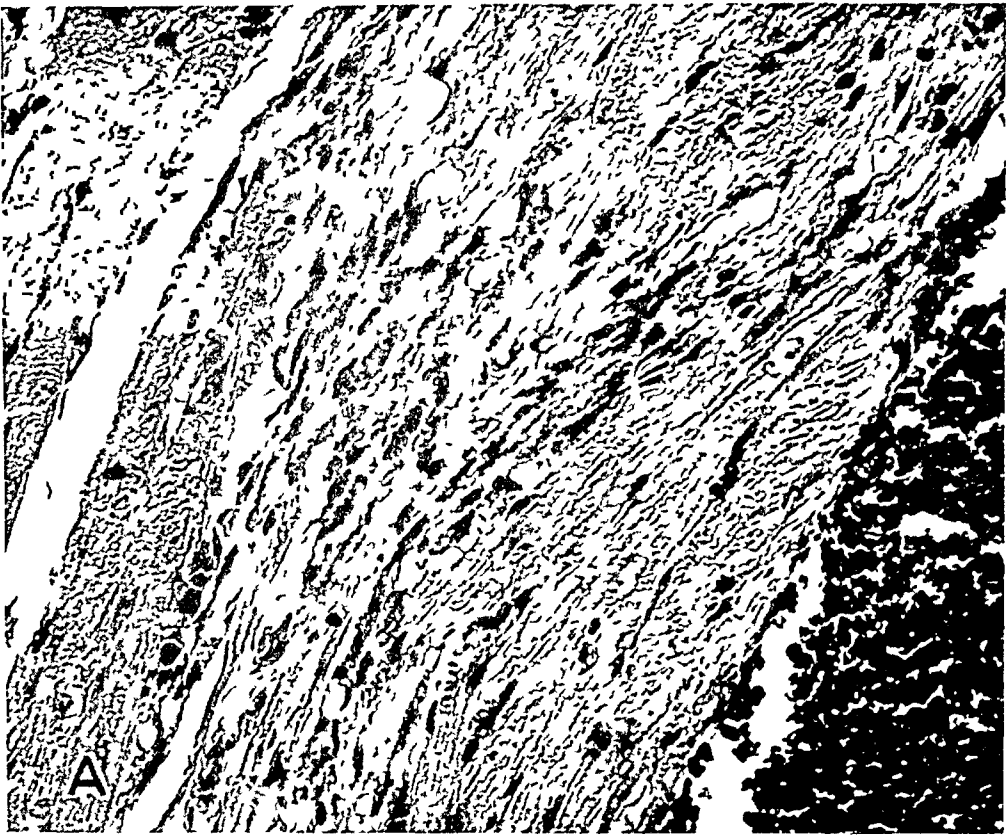


Fig 2—*A*, fibroblastic myocardial scar,  $\times 345$  *B*, myocardial arteriole with thickened, homogeneous wall and perivascular edema. The nuclei are reduced in number, markedly swollen and distorted. The localized crowding of nuclei is caused by an invasion of histiocytes into the degenerated and vacuolated media,  $\times 625$

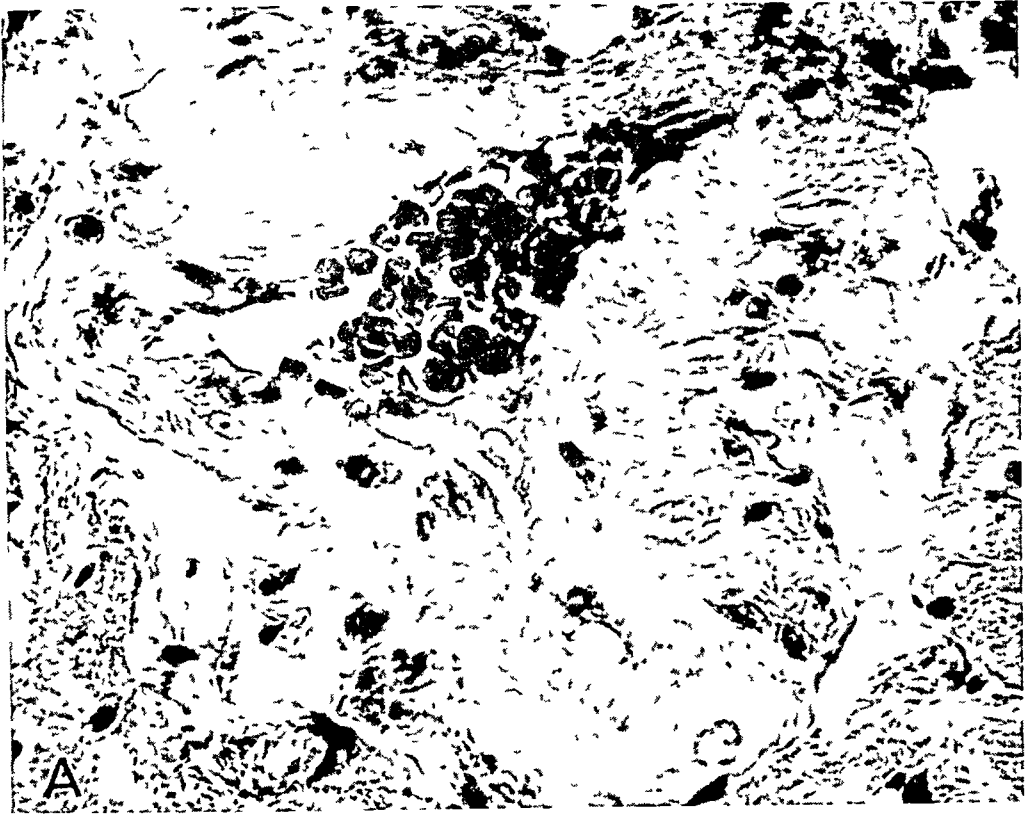


Fig 3—*A*, medium-sized myocardial artery with collapsed lumen and markedly thickened and hyalinized wall,  $\times 450$  *B*, large renal artery near the hilus with localized thickening and hyalinization of the wall,  $\times 230$  A few leukocytes and histiocytes are present in the marginal zone of the necrosis

(*d*) Stomach The gastric mucosa was normal in the rats of the two erythrol tetranitrate series, whereas it was congested and contained small hemorrhagic defects in the animals of the sodium nitrite series

(*e*) Liver The liver cells of most of the rats showed a peculiar fine granular or vacuolar degeneration of the cytoplasm. The nuclei often seemed to lie in empty spaces or to be located in a cytoplasm consisting of a loose accumulation of coarse granules

(*f*) Spleen The more or less markedly congested pulp contained in most instances a moderate to large amount of amorphous brown pigment located intracellularly and extracellularly. The brown granules stained greenish blue with the prussian blue method, while the delicate reticulum was impregnated with a dark blue iron-containing compound

(*g*) Kidney Frequently the tubular epithelium showed hyaline, granular degeneration and contained brownish globules which gave a negative reaction by the prussian blue method. Several of the large renal arterial branches at the hilus had a markedly thickened and locally hyalinized wall, the hyalinized area being demarcated from the intact smooth muscle cells of the media by small accumulations of mononuclear and lymphoid cells (fig 3 *B*). The walls of the renal arterioles were not infrequently markedly thickened and contained vacuolated and swollen smooth muscle cells and subendothelial and medial hyalinizations. The lumens were narrowed (fig 4 *A*). Several kidneys contained wedge-shaped areas in the cortex, consisting of a thickened interstitial tissue infiltrated with lymphocytes, atrophic and cystic tubules partly filled with a homogeneous, faintly calcified material and congested or atrophic and fibrotic glomeruli (fig 4 *B*)

(*h*) Testes In the testicular arteries of an appreciable number of rats calcified plates involved the media, occupying the entire circumference of the vessel. Some of these rats as well as some without the vascular changes showed more or less marked degenerative lesions of spermatogenic epithelium of the tubules. Spermatid giant cells and calcium incrustations in the tubular debris were seen in several instances

(*i*) Bone Marrow The marrow of the sternum and femur was often congested and was composed in the majority of cases of a dense myeloid tissue, in which the mature myeloid elements predominated. The erythropoiesis was usually moderate to marked. An immature marrow was seen in some instances

(*j*) Other Organs These were essentially normal with the exception of varying degrees of congestion

The recorded observations demonstrate that an appreciable number of the rats treated for varying periods with erythrol tetranitrate showed degenerative lesions in the myocardial arterioles. These were associated sometimes with regressive or histiocytic-fibroblastic changes in the myocardium. Occasionally, capillary hemorrhagic extravasations were seen in a congested and edematous myocardial interstitial tissue. A considerable proportion of the rats also exhibited calcifications of the subendothelial spaces and media of the arterial vessels of the lungs and testes. Arteriosclerotic lesions were not infrequently found in the kidneys. Regressive cellular changes were present in a large number of livers, especially among those from rats which survived for the entire experimental period. The spleens often showed evidence of chronic

passive congestion associated with destruction of erythrocytes, indicated by the large amount of iron-containing brown pigment found in many of these organs. In some of the testes extensive regressive changes

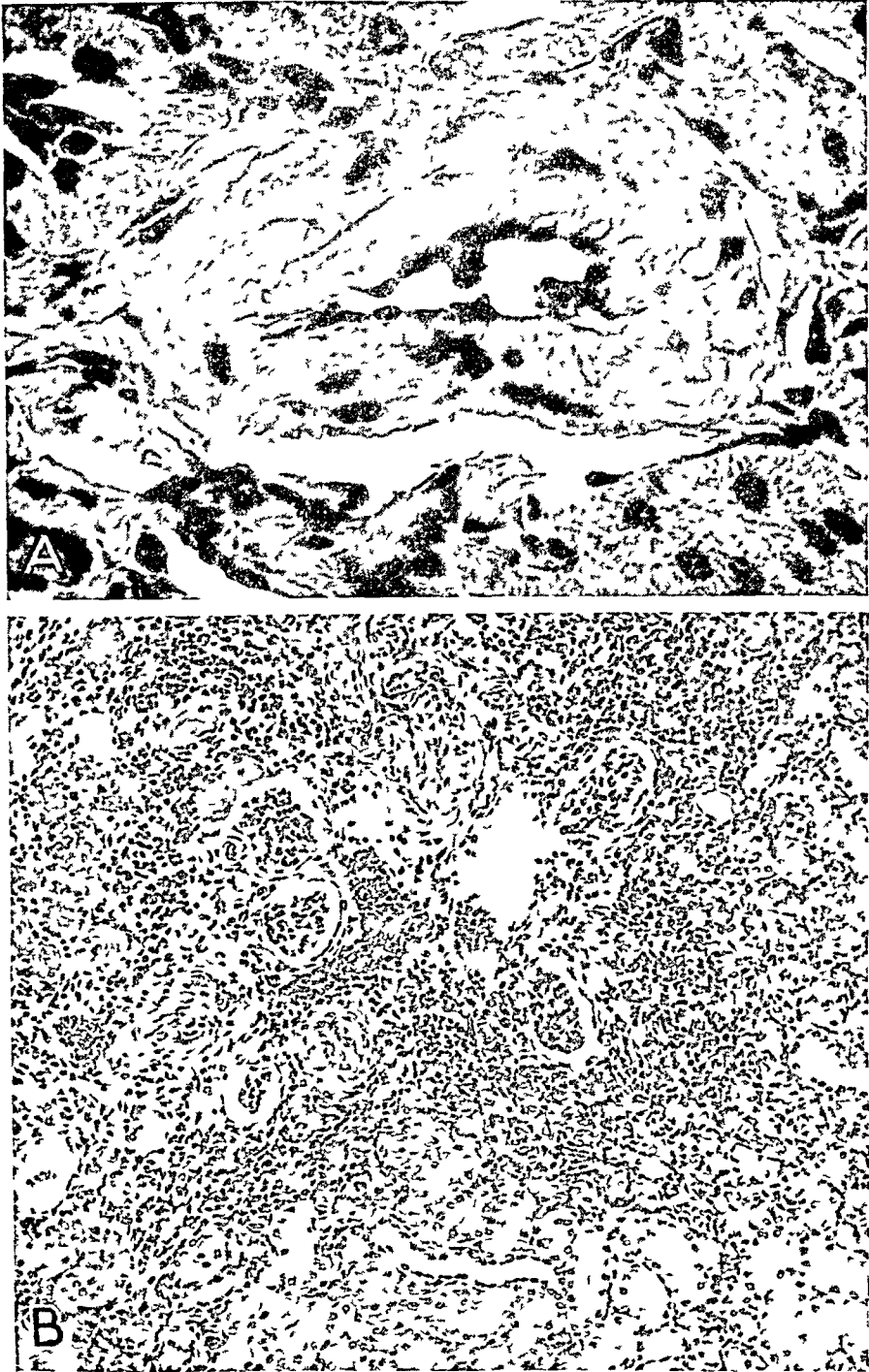


Fig 4—*A*, small renal arteriole with markedly thickened wall and narrowing of the lumen,  $\times 625$ . *B*, area of renal scarring with atrophic, fibrotic glomeruli, lymphocytic interstitial infiltration, fibroblastic proliferation, thickening of arteriolar walls, cystic distention of tubular lumens,  $\times 110$ .

of the spermatogenic epithelium were noted. The bone marrow in general displayed active erythropoiesis in the presence of an often rather mature but dense myeloid tissue. Hemorrhages and edema observed in a few cases, as well as hemosiderin in one instance, pointed to disturbances affecting both the circulation and the erythrocytes. The circulatory, vascular, glial and ganglionic changes found in the brains of an appreciable number of rats were a striking feature of the various pathologic lesions noted in these rats after the chronic introduction of erythrol tetranitrate by mouth. An analysis of these data in relation to the duration of exposure to this agent brought out the fact that the various pathologic lesions recorded increased in frequency and severity with the time of exposure.

TABLE 1—*Summation of Averaged Findings on the Blood of Rats After Chronic Oral Administration of Erythrol Tetranitrate*

Days of Experiment	Erythrocytes, Millions	Hemoglobin, Gm in 100 Cc	Mean Corpuscular Hemoglobin, Gm in 100 Cc	Leukocytes, Thousands
1	6.74	10.4	15	18.7
15	7.41	14.9	20	16.3
35	6.64	17.5	23	18.5
61	7.15	15.3	21	15.9
77	6.95	12.8	19	19.9
106	8.57	13.5	15	24.0
119	8.83	13.3	16	31.7
127	7.85	14.2	19	28.4
152	8.43	15.3	18	30.1
190	7.76	11.9	16	25.1
213	7.75	11.1	17	26.0
222	8.31	12.8	16	16.6

#### HEMATOLOGIC DATA

During the course of the experiment, studies were made on the blood of some of the rats of the two series which received erythrol tetranitrate. The hematologic examinations were started with 10 rats of the oral series (only 6 survived the experimental period) and an equal number of the subcutaneous series. The examinations were made at rather irregular intervals, which in general became longer with the duration of the investigation. The blood for the examination was obtained by cutting the tails. The blood constituents studied were the leukocytes (including the differential count), erythrocytes and hemoglobin. From the figures obtained for the two last mentioned elements, the value of corpuscular hemoglobin was calculated.

The results of the hematologic studies are presented in tables 1 and 2. The tables are constructed on the basis of averaged values obtained from the 6 and 10 rats, respectively, which could be followed through the entire period of nitrite treatment. This procedure was adopted because all the animals in a particular series showed the same trend. An analysis of the hematologic data shows the following facts:

*Oral Series*—The hemoglobin started to rise on the fifteenth day of the experiment and reached its peak of 17.5 Gm. on the thirty-fifth day. The values receded gradually during the rest of the experiment but always remained above the original level.

During the early period the erythrocyte counts fluctuated about a range within that given for normal rats<sup>5</sup> This was followed by a marked elevation, which reached the highest peak on the one hundred and nineteenth day with 8,830,000 cells Although the total red cell count dropped somewhat afterward, it fluctuated and maintained a mean definitely higher than the mean established for normal rats

The mean value of the corpuscular hemoglobin showed changes similar to those in the values of hemoglobin Following a rapid rise, which not only paralleled but surpassed the upward movement of the values of hemoglobin, there occurred a rather rapid drop in the amount of the corpuscular hemoglobin, which reached its lowest point on the one hundred and sixth day, when it was somewhat below the normal range The latter part of the experimental period was thus characterized by the fact that after marked fluctuations in the hemoglobin and the corpuscular hemoglobin values during the first part and considerable increases of

TABLE 2—*Summation of the Averaged Findings on the Blood After Subcutaneous Injection of Erythrol Tetranitrate*

Days of Experiment	Erythrocytes, Millions	Hemoglobin, Gm in 100 Cc	Mean Corpuscular Hemoglobin, Gm in 100 Cc	Leukocytes Thousands
1	7.23	16.2	22	18.7
3	6.67	14.7	22	14.4
5	6.53	13.2	19	14.5
9	6.03	12.5	21	13.8
14	5.52	11.2	20	26.4
20	4.91	11.5	24	15.6
36	6.16	9.7	18	30.3
57	6.53	11.8	16	29.0
73	8.07	13.0	16	38.9
79	6.56	12.9	20	34.1
86	7.12	9.0	13	35.7
107	6.72	11.1	17	34.5
126	7.47	9.9	13	42.5
133	7.31	11.0	15	22.1

the erythrocytes during the middle part, the three constituents became more or less stabilized toward the end, remaining somewhat above the original levels in a parallel movement

The total leukocyte count, after relatively minor fluctuations during the first two months of the experiment, showed a definite increase, which continued for the remaining five months The peak was reached on the one hundred and nineteenth day, when a maximum of 31,730 leukocytes was recorded From this period until almost the end of the experiment the total white cell count maintained itself at a level high above the normal range On the day that the rats were killed, however, the white cell count had dropped to 16,640 (twelve days after the discontinuation of the nitrite treatment)

The differential count did not show any significant shifts from the normal in spite of the considerably increased number of leukocytes present during the latter half of the experimental period

It was observed that in most instances the number of reticulocytes was increased definitely after the administration of erythrol tetranitrate, while the number of platelets exhibited a tendency toward lower values, within the normal range, during the latter part of the experimental period

<sup>5</sup> Wintrobe, cited by Cushny<sup>6</sup>



*Subcutaneous Series*—The values of hemoglobin dropped almost steadily from the start of the experiment, reaching their lowest figure (9.76 Gm) at the end of the fifth week. Following a transitory rise, the values dropped again and reached their lowest figure (9 Gm) at the end of the third month. There was subsequently a moderate rise with values somewhat below the normal range (i.e., between 9.9 and 12.9 Gm).

The erythrocyte count exhibited a similar initial reduction, which was also followed by a gradual rise. This, however, was more prolonged than that of the values of hemoglobin. After having reached a figure slightly above the original one, the erythrocyte count decreased again and remained for the rest of the experimental period within normal limits.

Following a slight initial drop and a subsequent steep rise to somewhat above the normal level, the corpuscular hemoglobin decreased severely and persistently and remained at a reduced level for the last part of the experiment.

The leukocytes remained stationary as to number and type during the first quarter of the experimental period, then rose constantly until they reached their peak before the end of the experiment, when 42,470 white cells represented the average leukocyte count. There was at this time a moderate increase in the proportion of the neutrophilic and especially of the juvenile leukocytes.

#### COMMENT

The significance of the recorded vascular lesions and of the parenchymatous organic alterations depends on the demonstration of causative interrelations between the morphologic phenomena and the administration of erythrol tetranitrate and sodium nitrite, respectively. Inasmuch as Hueper,<sup>1</sup> Wilens and Sproul<sup>2</sup> and Hummel and Baines<sup>3</sup> reported the spontaneous occurrence of similar cardiovascular lesions in rats, it is essential that satisfactory evidence be supplied which will exclude a possibly senile character of the organic changes observed in the rats treated with nitrites. The observations made by Wilens and Sproul<sup>2</sup> on the age distribution of these pathologic changes of the circulatory tissue of rats are therefore of great importance. These investigators found that rats less than 400 days old are practically free from degenerative cardiac lesions, while the coronary sclerotic changes noted in 23 of 487 rats examined showed in their age incidence a direct relation to the senile period. Similar observations were made by Hummel and Baines, who noted that sclerotic cardiovascular lesions were found in only 2 of 111 rats dying at an age of 261 to 374 days. As the animals comprising the nitrite series were in no instance older than 300 days, i.e., they belonged to an age period during which normally degenerative cardiovascular changes are rare or absent, it becomes obvious that the organic lesions observed cannot be considered as the physiologic accompaniments of old age but must be regarded as the results of the nitrite treatment. This conception is supported by the fact that they occurred in the rats not only with marked severity and high incidence but with increasing frequency corresponding to the duration of exposure to the nitrites.

A possible senile nature of the lesions observed in the treated rats having been excluded, it becomes pertinent to demonstrate whether the symptomatic and morphologic manifestations noted in the experimental animals resulted from the pharmacologic and toxicologic effects known to be exerted by nitrites and whether these reactions were similar to or identical with those seen in chronic nitrite poisoning of man

The functional effects which inorganic nitrites as well as inorganic and alkyl nitrates exert on the vascular walls and the circulation of the blood are expressed in a relaxation of the walls of the peripheral arterioles with a resultant drop in blood pressure. The most susceptible vessels, which react first and to the smallest doses, are those of the head, neck, brain and meninges. Larger doses cause relaxation of the coronary vessels, while still larger ones produce dilatation of the vessels of the splanchnic region. It is this widening of the vascular bed which causes the drop in blood pressure. The slowing of the blood flow results in congestion, which in turn is followed by development of hypoxemic states, especially in those organs in which the demand for oxygen is high or which are highly susceptible to even minor deficiencies in the oxygen supply (brain and heart). It has been noted that the degree of vasodepression elicited depends not only on the particular type of nitrite used and the dose given but to a great extent on the subject as well as on his age (Cushny<sup>6</sup>, Lowy<sup>7</sup>, Prodger and Ayman<sup>8</sup>, Sprague and White<sup>9</sup>)

The ensuing subjective symptoms of vasculocirculatory disturbances observed in chronic nitrite poisoning are: transitory or more or less persistent low blood pressure, which may drop below 80 mm of mercury, a weak, soft pulse, dilatation of the left side of the heart, accentuation of the first heart sound, exaggeration of the apical beat, murmurs similar to those found with anemia, aortic regurgitation or mitral insufficiency, irregularity of the heart beat, and other signs of myocardial impairment. Tachycardia or bradycardia is often present, the latter condition, however, changing readily into tachycardia on slight physical exertion, excessive change in environmental temperature and humidity or consumption of alcoholic beverages or other agents which exert an additional vasodepressant effect. Precordial pain and anginoid attacks are not infrequently recorded. During more acute episodes the color

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6 Cushny, A. R. Die Nitritgruppe, in Heffter, A. Handbuch der experimentellen Pharmakologie, Berlin, Julius Springer, 1923, vol. 1, p. 833, A Text Book of Pharmacology and Therapeutics, ed. 11, Philadelphia, Lea & Febiger, 1936, p. 630

7 Lowy, J. Die Klinik der Berufskrankheiten, Vienna, Emil Haim & Co., 1924, p. 392

8 Prodger, S. H., and Ayman, D. Am J M Sc **184** 480, 1932

9 Sprague, H. B., and White, P. B. M Clin North America **16** 895, 1933

of the skin is purplish livid, most noticeable in the nail beds, lips, ears and nose. As the result of the direct or indirect action of the nitrites on the cerebral circulation there occur, in addition to headache, diverse symptoms such as insomnia, restlessness, maniacal episodes, states of drowsiness and mental dulness. The vasodepressant effect on the splanchnic system causes the appearance of symptoms of indigestion and gastroenteritis (colic, bloody diarrhea) resulting from chronic congestion and hemorrhages of the gastric and intestinal mucosa. Sudden death may ensue with the symptoms of cerebral paresis (Meixner and Mayrhofer<sup>10</sup>) or of coronary thrombosis (International Labor Office Report<sup>11</sup>, Lowy<sup>7</sup>, Laws<sup>12</sup>, Ebright<sup>13</sup>, Robert<sup>14</sup>, Schulz,<sup>15</sup> and others).

The following macroscopic and microscopic lesions have been recorded in connection with deaths from nitrite poisoning: meningeal and cerebral edema and congestion with perivascular edema and accumulation of lymphoid and glia cells and brown pigment, multiple cerebral hemorrhages affecting mainly the region of the brain stem, intimal proliferation, medial hyalinization, swelling and calcification of the cerebral vessels, perivascular fibrous scars with capillary proliferation, gliosis and blanching of the nerve substance, glial scars, acute and chronic degeneration of ganglion cells, involving chiefly the centers of the brain stem, coronary sclerosis, swelling and hyalinization of the walls of the myocardial arterioles, enlargement of the heart with hypertrophy of the left ventricular wall, myocardial degeneration, fibrosis and cicatrization, chronic passive congestion of the liver with pericentral fatty degeneration, hemorrhagic gastritis and enteritis, nephrosis, arteriosclerosis of the renal vessels, and pancreatic hemorrhages (Solis-Cohen and Githens<sup>16</sup>, Lowy<sup>7</sup>, von Jaksch<sup>17</sup>, Binz<sup>18</sup>, Meixner and Mayrhofer<sup>10</sup>, Fischer-Wasels,<sup>19</sup> and others).

During the course of the experiment several of the listed symptomatic manifestations of chronic nitrite poisoning, such as cold livid skin, mental depression, aggressiveness, as well as fainting spells, and attacks of tachycardia, were observed following the injection of nitrite. The

10 Meixner, K., and Mayrhofer, A. *Vrtljschi f gerichtl Med* **61** 228, 1921

11 International Labor Office Reports, vol 2, no 281, Geneva, Switzerland International Labour Office

12 Laws, G. C. *J. A. M. A.* **31** 793, 1898

13 Ebright, G. E. *J. A. M. A.* **62** 201, 1914

14 Robert, E. *J. de med. de Bordeaux* **112** 10, 1935

15 Schulz, O. *Samml. f. Vergiftungsfälle (B)* **6** 59, 1935

16 Solis-Cohen, S., and Githens, T. S. *Pharmacotherapeutics*, New York D. Appleton and Company, 1928, p. 370

17 von Jaksch, cited by Lowy<sup>7</sup>

18 Binz, C. *Arch. f. exper. Path. u. Pharmacol.* **13** 133, 1881

19 Fischer-Wasels, B. *Frankfurt Ztschr. f. Path.* **45** 1, 1933

hematologic findings reflected the changes produced in the circulation and oxygenation of the blood. The hematologic effects were especially striking in the oral group. Following a primary compensatory phase of hemoglobinosiis, there developed an appreciable and relatively continuous erythrocytosis in response to the persistent and possibly intensified circulatory hypoxemia produced by the administration of nitrites. Confirmatory evidence of the presence of marked circulatory embarrassment during the latter part of the experimental period in both series is furnished by the behavior of the leukocytes. The considerable leukocytosis without the usually occurring shift toward a higher proportion of immature cell forms and with the presence of a predominantly mature or resting myeloid marrow characterizes the leukocytotic condition as a distributory phenomenon representing leukocytostasis in the distal peripheral vessels, such as those of the tip of the tail, caused by excessive slowing of the blood flow.

The anatomic studies of the organs of the rats treated with nitrites revealed that the vascular and parenchymatous lesions produced in these young animals were identical with those observed in human subjects who had been exposed and subsequently succumbed to a chronic exposure to chemically related nitrites and similarly acting nitrates. It is obvious that the existence of causal relations between the regressive and sclerotic vascular alterations and some of the related parenchymatous degenerations (especially those in the brain, heart, kidney and testis) and the hypotensive and hypoxemic effect exerted by the nitrite has not been recognized in the past because the anatomic manifestations are very similar to those considered representative of, and resulting from, or accompanying, chronic hypertension. In the absence of adequate etiologic and symptomatic data these pathologic changes may have been misinterpreted or may have been considered as coincidental.

It is believed that the experimental data presented offer satisfactory evidence in support of the actual existence of such interrelations and thus furnish experimental proof of the original premise that arteriosclerotic lesions may develop on a hypotensive as well as on a hypertensive basis, provided the hypotensive condition is sufficiently severe and prolonged. While an inordinate hypertensive vasoconstriction interferes with proper nutrition of the vascular wall, especially of its inner portion, not supplied with vasa vasorum, by a reduction of the quantity of blood which can penetrate these more or less compressed vessels (ischemic hypoxemia), the same effect is produced by the stagnant hypoxemia resulting from the excessive slowing of the blood flow accompanying hypotensive vasodilation, which also affects mainly those parts of the vascular walls in which vital activity depends on nutritive exchange and discharge of waste products through the process of diffusion.

## SUMMARY

Oral and subcutaneous administration of erythrol tetranitrate and sodium nitrite, respectively, to young, immature rats over a period of several months results in the production of degenerative vascular and parenchymatous lesions in the heart, lung, brain, kidney and testis

The chief causative factor in the development of these changes is represented by a stagnant hypoxemia resulting from the slowing of the blood flow caused by the hypotensive vasodilatation elicited by the nitrite

These experimentally produced organic changes are identical with those seen in man following chronic nitrite poisoning and are very similar to those observed in spontaneous or experimental hypertension as found in man and animals, respectively

Inasmuch as vasodilating agents as well as vasoconstricting agents cause a nutritive disturbance of the vascular walls, prolonged vasodilatation or vasoconstriction (hypotension or hypertension) results in the appearance of degenerative and reactive proliferative phenomena in the vascular walls, which are considered as characteristic of arteriosclerosis and arteriolosclerosis, angionecrosis and atheromatosis

The same causative mechanisms produce, by hypoxemia and associated nutritive disturbances, regressive changes in the parenchymatous organs (heart, brain, kidney, testis), which are accentuated by the progressive circulatory impairment resulting from the vascular changes

# INCIDENCE OF PRIMARY CARCINOMA OF THE LUNG

A REVIEW OF YALE AUTOPSY PROTOCOLS, 1917 TO 1937

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In an earlier report<sup>1</sup> on the incidence of primary carcinoma of the lung it was concluded that the generally recognized increase in the frequency of this neoplasm was real and absolute. Excellent general reviews, particularly by Fried<sup>2</sup> and by Simons,<sup>3</sup> have since been published, but there still exists marked divergence of opinion as to the nature of the observed increase in frequency of bronchiogenic carcinoma. Is this increase apparent or real, relative or absolute? After analyzing the various factors involved, Fried<sup>2</sup> concluded that "the increase then is very likely more apparent than real," and Frissell and Knox<sup>4</sup> concurred in this belief, stating that "it would seem probable that the increase in the incidence of bronchial carcinoma in the last two decades is apparent rather than real." On the other hand, Hill,<sup>5</sup> after a survey of the relevant factors, declared that "a real increase in the incidence has occurred," and more recently Matz<sup>6</sup> indicated his opinion that "the increased incidence of bronchiogenic carcinoma is absolute."

The problem at first glance may appear to be only of academic importance. Its resolution, however, has broad implications. If, as some believe, the increase in the number of pulmonary cancers is merely an expression of the aging of a population subjected to improved diagnostic facilities and sharper clinical acumen, little can be done by way of prophylaxis. On the other hand, if the increase is greater than can be expected on the basis of increasing numbers of persons in the older age groups, greater even than can be ascribed to more accurate diagnostic methods, attention can be directed to the elucidation and perhaps ultimate elimination of the responsible factor or factors. It is the purpose

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1 Rosahn, P D. *Am J M Sc* **179** 803, 1930

2 Fried, B M. *Medicine* **10** 373, 1931

3 Simons, E J. *Primary Carcinoma of the Lung*, Chicago, The Year Book Publishers, Inc., 1937

4 Frissell, L F, and Knox, L C. *Am J Cancer* **30** 219, 1937

5 Hill, R M. *Edinburgh M J* **41** 320, 1934

6 Matz, P B. *J A M A* **111** 2086, 1938

of this paper to present new data on the subject together with a critical analysis of the reasons generally advanced for accepting the view that the increase in the incidence of carcinoma of the lung is only apparent. Pertinent data of correlative interest are also presented.

#### MATERIAL AND METHODS

The first autopsy in the current series at the Yale University School of Medicine was performed on Sept 22, 1917. From this date to Dec 31, 1937 a total of 4,156 persons were examined post mortem. The cranial cavity only was examined in 42 of these, and, of the remaining 4,114, 1,887 were males over 20 years of age and 1,081 were females over 20. The latter two groups, totaling 2,968 persons, form the basis of the present report. In the discussion, the period from Sept 22, 1917 to Dec 31, 1927 has been termed the first decade, and the period from Jan 1, 1928 to Dec 31, 1937 has been called the second decade.

The age and sex of every person with carcinoma coming to autopsy, together with the primary site, were recorded. In the vast majority of these persons the neoplasm was the primary cause of death, but in a small number carcinoma was an incidental finding at autopsy. Statistical constants were then determined for each organ or system involved, and the annual and decennial incidence calculated. For comparative purposes the age and the sex distribution of all persons in the entire autopsy series aged 20 years or older were also tabulated and statistical constants calculated. For this purpose the punched card system described elsewhere<sup>7</sup> proved of inestimable assistance.

The statistical methods employed were those which are in common use, and the texts of Fisher<sup>8</sup> and Snedecor<sup>9</sup> were freely consulted. When the probability of an event occurring by chance alone was 5 or less than 5 in 100 trials ( $P \leq 0.05$ ) the occurrence of the event was said to be *significant*. If the event could occur by chance once or less than 1 in 100 trials ( $P \leq 0.01$ ), the event was said to be *highly significant*. This is in accordance with Snedecor's terminology.

A new concept in time trend regression equations is here introduced. The accepted method of analyzing a trend in time involves the use of equal intervals of time as the independent variable. As applied to the question under discussion, the incidence of primary carcinoma of the lung, the orthodox procedure would be to plot the proportion of primary pulmonary cancers to all cancers occurring in succeeding equal time intervals. An error is thus introduced, its magnitude depending on the size of the sample. For it is self evident that 2 cases of tumor of the lung among 20 instances of carcinoma are of less significance than 6 cases among 60 instances of carcinoma, although the resulting percentages are the same in each instance. When the total number of tumors per successive unit of time varies, the error of the ratio between one type of tumor and all types fluctuates correspondingly. Moreover, successive determinations in the problem at hand need not necessarily be made on the basis of equal time intervals. What interests the observer in this connection is whether one type of tumor is changing in frequency over a given period of time. The broad vista of this period concerns him, and not the equality of the successive intervals composing it.

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7 Rosahn, P. D. J. Tech. Methods **18** 32, 1938.

8 Fisher, R. A. Statistical Methods for Research Workers, ed 5, London, Oliver & Boyd, 1934.

9 Snedecor, G. W. Calculation and Interpretation of Analysis of Variance and Covariance, Ames, Iowa, Collegiate Press, Inc., 1934.

In considering this problem it appeared reasonable to relate the incidence of carcinoma of any organ to successive equal numbers of all cancers without regard to the time period during which these equal numbers of cancers were observed. This procedure would thus eliminate the variable error resulting from the occurrence of different numbers of tumors in successive equal intervals of time. Following this reasoning, all carcinomas were numbered in sequence as they were found in the autopsy protocols, and the regional distribution of the carcinomas comprising the first, second, third and fourth hundred carcinomas was then determined. Thus the error of a proportion was eliminated, and yet the use of one hundred as a measuring rod proved to be of great convenience, since it permitted a direct translation from the absolute number of carcinomas of any one region per hundred of all carcinomas into the corresponding relative percentage.

## RESULTS

A total of 435 carcinomas occurring in 425 different persons was observed in the series of 4,114 autopsies. The regional distribution of

TABLE 1—*Regional Distribution of Carcinomas Observed in Yale University Autopsy Series, 1917-1937*

Organ Involved	1917-1937		1917-1927		1928-1937	
	Number	Percentage	Number	Percentage	Number	Percentage
Large intestine	71	16.3	21	15.4	50	16.7
Stomach	58	13.3	27	19.9	31	10.4
Lung	43	9.9	10	7.4	33	11.0
Prostate	32	7.4	5	3.7	27	9.0
Uterus	29	6.7	13	9.6	16	5.4
Esophagus	27	6.2	8	5.9	19	6.4
Breast	26	6.0	8	5.9	18	6.0
Liver and bile ducts	26	6.0	12	8.8	14	4.7
All others	123	28.3	32	23.5	91	30.4
Total	435	100.1	136	100.1	299	100.0

these neoplasms for each ten years and for the entire twenty year period is shown in table 1. During the interval from 1917 to 1927 there were 10 primary pulmonary tumors, or 7.35 per cent of the 136 carcinomas encountered. This compares with 33 primary pulmonary cancers seen in the period from 1928 to 1937, or 11.04 per cent of the 299 cancers observed. It is of interest to note that the relation of pulmonary cancers to all cancers during the period 1917 to 1927 in this report is similar to the findings<sup>1</sup> at the Boston City Hospital during the nearly comparable period from 1920 to 1928. 7.35 per cent in the first instance and 7.23 per cent in the second. Both of these values correspond closely to the 6.98 per cent incidence of primary carcinoma of the lung among all carcinomas found in a review<sup>1</sup> of the world literature for the period from 1920 to 1928.

The table indicates that during the period from 1917 to 1927 carcinoma of the lung was the fifth most frequent tumor, being exceeded in frequency by carcinoma of the stomach, large bowel, uterus and liver and bile ducts, in this order. In the following decade carcinoma of the stomach fell to third position, and carcinoma of the lung rose to second



place, being exceeded in frequency only by malignant tumors of the large bowel. It will be seen that with the exception of prostatic carcinoma, carcinoma of the lung was the only neoplasm the incidence of which increased appreciably during the second of the two ten year periods under consideration. As will be shown in a subsequent table, the increase in the incidence of prostatic carcinoma was variable, and the trend line for this tumor showed no significant deviation from zero.

Table 2 gives the regional distribution of the neoplasms comprising the first, second, third and fourth hundred carcinomas. The first hundred carcinomas were observed during the ten year period from October 1917 to September 1926, the second hundred in the three and a half year period ending March 1930, the third hundred during the nearly four year period ending January 1934 and the fourth hundred

TABLE 2—*Regional Distribution of First to Fourth Hundred Carcinomas*

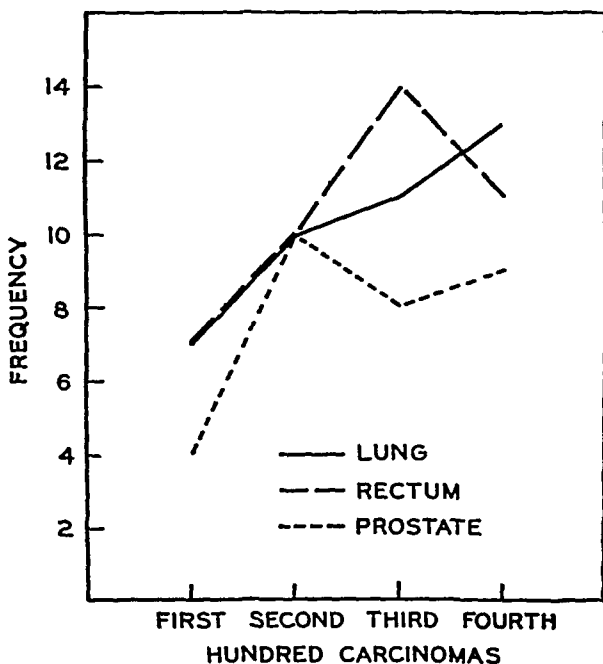
Carcinomas	Period of Observation	Site								Total
		Stomach	Rectum and Sig-moid	Lung	Prostate	Uterus	Liver and Bile Ducts	Esophagus	All Others	
1-100	October 1917 September 1926	21	7	7	4	9	6	7	39	100
101-200	September 1926 March 1930	12	10	10	10	6	9	6	37	100
201-300	March 1930 January 1934	14	14	11	8	4	7	1	41	100
301-400	January 1934 December 1936	9	11	13	9	9	4	8	37	100
1-400	October 1917 December 1936	56	42	41	31	28	26	22	154	400

during the following two year interval ending December 1936. Thirty-five additional carcinomas noted between Dec 15, 1936 and Dec 31 1937 are not included in this table because they did not fit conveniently into the "one hundred carcinoma" grouping followed. The variability of the time intervals during which successive hundred carcinomas were seen is largely a reflection of the increasing annual number of autopsies performed. Only 7 persons with primary carcinoma of the lung were encountered in the period from 1917 to 1926, during which the first hundred carcinomas were encountered at autopsies. The number progressively increased to 13 in the interval from 1934 to 1936, when the fourth hundred carcinomas were observed. In the last period primary carcinoma of the lung was the most frequent of all tumors. The increasing incidence is shown graphically in the chart, which for comparative purposes shows the trends of carcinoma of the prostate and rectum during comparable periods.

The trend line for the frequency of carcinoma of the lung in each of the four successive hundred carcinomas is represented by the regres-

sion equation  $Y' = 5.5 + 1.9X$ , with  $S_{\pi}$  being equal to 0.6, and  $t$ , to 5.51. The coefficient of correlation ( $r$ ) between the number of primary pulmonary carcinomas and the succeeding hundred carcinomas was  $+0.9744$ . The foregoing values of  $t$  and of  $r$  are both within the range of significance. Such a trend as that described would be expected to occur by chance alone less than four times in a hundred similar series.

The significance of the increasing incidence of primary cancer of the lung is further enhanced by the demonstration that it alone of all tumors had a significant positive trend. Regression equations prepared in the same manner for the incidence of carcinoma of the stomach, liver and



Frequency of carcinoma of the lung, rectum and prostate, respectively, in the first to fourth hundred carcinomas in the Yale autopsy series

bile ducts, prostate, intestine, female genitalia, esophagus and rectum, respectively, all showed no significant deviation from the horizontal

#### ANALYSIS OF CERTAIN FACTORS OFTEN CITED AS EVIDENCE THAT CANCER OF THE LUNG HAS SHOWN ONLY A RELATIVE INCREASE

The arguments in favor of the belief that the generally reported increase in the incidence of primary carcinoma of the lung is relative and not absolute have been summarized best by Fried

The author is of the opinion that the more frequent occurrence of this disease in recent years, as compared to the older findings, can be explained on the basis of the following factors (1) Improved clinical and pathological methods of

diagnosis, (2) Increased attention to this malady (as Goethe has expressed it, "Man sieht nur was man weiss"), (3) Increase in span of human life (a much greater proportion of people reach the "cancer age")

Frissell and Knox have added a fourth reason, also suggested by Fried

Since external and readily diagnosed cancer has not increased, it would seem probable that, in spite of the evidence of the post-mortem figures, especially in Germany, the increase in the incidence of bronchial carcinoma in the last two decades is apparent rather than real

Each of these factors will be discussed, the material here presented being used as a background

1 *Improved Clinical and Pathologic Methods of Diagnosis*—There is no doubt that the past decade has seen marked technical advances in diagnostic facilities and that the clinical diagnosis of carcinoma of the lung has been made more frequently than previously. The use of iodized poppyseed oil in conjunction with roentgenographic work, the greater knowledge of bronchoscopy and wider employment of the bronchoscope together with the thoracoscope and lung puncture have all added to the frequency of the antemortem diagnosis of carcinoma of the lung. The records of the New Haven Hospital have recently been reviewed by Kober,<sup>10</sup> who found 26 cases in which primary cancer of the lung had been demonstrated by biopsy without autopsy. The first tumor in this series was diagnosed in 1921. Half of the 26 tumors were diagnosed ante mortem during the fourteen year period from 1921 to 1935, while the remaining half were diagnosed during the two years 1936 and 1937. It should be emphasized that none of these 26 tumors are included in the present report, which is based solely on autopsy material. The increase in the number of pulmonary cancers diagnosed ante mortem enhances the value of the statistically significant increase in the postmortem incidence. Moreover, this postmortem increase, credited by some to be predominantly German, has also been widely recognized in this country.

Changes have also occurred in the histologic classification of primary pulmonary tumors. As Frissell and Knox<sup>4</sup> stated

First, many tumors classified as sarcomata by pathologists of the last century are now included as epithelial tumors of the so-called oat-cell variety. Secondly, carcinoma of the lung, when found, was usually considered metastatic. Third, the widespread interest of pathologists in this subject, particularly in the last decade, has led to the discovery of a considerable number of small pulmonary neoplasms with large metastases, such metastases earlier observers undoubtedly regarded as the primary lesion.

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<sup>10</sup> Kober, W. M. Primary Carcinoma of the Lung, Thesis, Yale University School of Medicine, 1938.

My co-workers and I have reviewed our cases with these three points in mind. In only 2 instances was the original anatomic diagnosis altered after restudy of the slides. The autopsy in the first of these cases (A 1443) was done in 1927, and the tumor was originally called a "malignant mediastinal neoplasm." This on reexamination was found to be a typical bronchiogenic carcinoma of the oat cell variety. In the second instance (A 1705) the autopsy was made in 1928, and at that time the tumor was classified as a "squamous cell carcinoma involving pericardium, pleura and thoracic wall." On restudy this was included as a squamous cell carcinoma of the lung. Both of these tumors were in the second hundred carcinomas. Had the original diagnoses been accepted and had the tumors as a result been excluded from the series, the resulting regression equation would have had greater significance,  $t$  in this case being equal to 6.05 ( $P = 0.03$ —). Even though these 2 tumors were included, however, the calculated regression equation retained a significant positive deviation from zero. In this connection it should be stated that all mediastinal tumors occurring in the period under discussion were reviewed, and the aforementioned one (A 1443) was the only tumor reclassified as a primary carcinoma of the lung. In none of the other cases mentioned, with the exception of A 1705, was there any doubt that the lung was the primary site of the neoplasm.

*2 Increased Attention to This Malady*—This statement appeared in 1931 to explain the increasing incidence of primary pulmonary tumors noted up to that time. There can be little doubt that a certain number of tumors diagnosed carcinoma of the lung in the past twenty years would previously have been assigned to some other diagnostic category not only by the clinician but by the pathologist also. But to explain the statistically significant increase in the number of pulmonary cancers in our autopsy material of the past twenty years on the basis of increased attention to this malady presupposes a constantly increasing awareness of this condition on the part of both clinician and pathologist from 1917 to 1931 and thereafter to the present. The curve of attention to carcinoma of the lung would thus have to show a positive linear trend paralleling the increasing incidence of pulmonary tumors. It would be necessary to assume also that in the years under discussion the attention to and interest in tumors of other organs were maintained at a constant level. Neither of these hypotheses is susceptible of proof, and they do not appear plausible. Since the number of publications on a subject is an excellent indication of the general interest in that subject, the *Index Medicus* for the past twenty years was examined. The number of published reports on various aspects of carcinoma has increased tremendously during this period, but what is of more importance in the present connection is the fact that carcinoma of practically every organ,

and not of the lung alone, is represented by an increased number of publications

In this connection, it has been said that because patients with carcinoma of the lung present peculiar diagnostic difficulties, intensive efforts are expended to secure consent for necropsy in such cases. As a result, it is claimed, a greater proportion of persons with tumor of the lung come to autopsy than would otherwise be the case, accounting in part for the recorded increase in the incidence of this neoplasm. However, the difficulties involved in the diagnosis of carcinoma of the lung are probably no greater than those accompanying the diagnosis of a suspected neoplasm of any other visceral organ. The symptom complex of carcinoma of the lung is perhaps no more bizarre and no more challenging from a diagnostic point of view than a malignant tumor of the liver and bile ducts. Nevertheless, tumors of the latter system in the reported series did not increase in number during the same period when

TABLE 3—*Mean Age of Persons Over Twenty Years of Age in Yale University Autopsy Series, 1917-1937*

Period	Total Series		Women *		Men †	
	Number	Mean Age, Yr	Number	Mean Age, Yr	Number	Mean Age, Yr
1917-1927	946	49.9 ± 0.59	359	50.1 ± 0.97	387	49.7 ± 0.65
1928-1937	2,004	53.5 ± 0.37	719	53.4 ± 0.62	1,285	53.5 ± 0.46
Total	2,950	52.3 ± 0.32	1,078	52.3 ± 0.76	1,872	52.3 ± 0.38

\* Three women of "unknown age" were excluded

† Fifteen men of "unknown age" were excluded

an increase in the incidence of carcinoma of the lung occurred. Were the increase in pulmonary tumors at autopsy dependent on the zeal of the hospital staff in obtaining permission for necropsy because of difficulties in diagnosis, it appears that a similar increase in tumors of the liver and bile ducts should also have been observed.

3 *Increase in the Span of Human Life*—An analysis of the ages of persons over 20 who have come to autopsy reveals an increase in the mean age of both males and females in the period from 1928 to 1937 as contrasted with the period from 1917 to 1927. This is shown in table 3. It will be recalled that the first hundred carcinomas were noted during the period from 1917 to 1926, which nearly coincides with the first decade of this review. The mean age of men increased from  $49.7 \pm 0.65$  years in the first of these two decades to the significantly higher level of  $53.5 \pm 0.46$  years in the second decade. At the same time the mean age of women showed a significant increase from  $50.1 \pm 0.97$  years to  $53.4 \pm 0.62$  years. Men and women had the same mean age in the period from 1917 to 1927, and again the same mean age, but at a higher level, in the period from 1928 to 1937.

The "increase in the span of human life" is thus reflected in our own autopsy experience. Nevertheless, the incidence of carcinoma when standardized for age and sex failed to reveal any increase. Tables 4 and 5 illustrate this finding. Of interest is the fact that the incidence of carcinoma in each age group and in both sexes was generally higher in the period from 1917 to 1927 than in the period from 1928 to 1937. The outstanding exceptions occurred among women aged 70 and over,

TABLE 4—*Incidence of Carcinoma Among Women Over Twenty Years of Age*

Age	1917-1937			1917-1927			1928-1937		
	Num ber	No with Carcinoma	Per centage	Num ber	No with Carcinoma	Per centage	Num ber	No with Carcinoma	Per centage
20-29	148	2	1.4	67	0	0	81	2	2.5
30-39	184	15	8.2	55	6	10.9	129	9	7.0
40-49	193	26	13.5	62	9	14.5	131	17	13.0
50-59	217	43	19.8	56	18	32.1	161	25	15.6
60-69	183	37	20.2	61	16	26.2	122	21	17.2
70 plus	153	30	19.6	58	10	17.2	95	20	21.1
Unknown	3	0	0	2	0	0	1	0	0
Total	1,081*	153	14.2	361	59	12.4	720	94	13.1

\* One woman under 20 with carcinoma was excluded from this analysis.

TABLE 5—*Incidence of Carcinoma Among Men Over Twenty Years of Age*

Age	1917-1937			1917-1927			1928-1937		
	Num ber	No with Carcinoma	Per centage	Num ber	No with Carcinoma	Per centage	Num ber	No with Carcinoma	Per centage
20-29	189	1	0.5	69	0	0	120	1	0.8
30-39	228	10	4.4	103	8	7.7	125	2	1.6
40-49	419	45	10.7	139	15	10.8	280	30	10.7
50-59	413	81	19.6	117	25	21.4	296	56	19.0
60-69	372	74	19.9	100	15	15.0	272	59	21.7
70 plus	251	59	23.5	59	10	16.9	192	49	25.5
Unknown	15	1	6.7	8	1	12.5	7	0	0
Total	1,887	271	14.4	595	74	12.4	1,292	197	15.2

and among men aged 60 and over, in these age groups the incidence of cancer was *greater* in the period from 1928 to 1937 than in the previous ten year experience. The combined values for the incidence of carcinoma in persons coming to autopsy, however, revealed no significant difference between the sex groups or between the two ten year periods of the survey. The incidence of carcinoma among persons over 20 years of age was 14.3 per cent, time and sex having exerted no significant influence on this value.

The material has been subjected to a further analysis with a view to ascertaining what effect, if any, the increased span of life had on the

age of persons with carcinoma at autopsy Table 6 shows the mean age of men and of women with carcinoma in the two ten year periods of the review The mean age of men for the twenty year period was  $60.48 \pm 0.69$  years, which is significantly higher than the comparable age,  $57.47 \pm 1.10$  years, for women ( $D = 3.01 \pm 1.29$ ,  $T = 2.34$ ,  $P = 0.02$ —) The age of men dying with carcinoma in the period from 1917 to 1927, the age of women dying in the same period and the age of women dying in the period from 1928 to 1937 did not differ signifi-

TABLE 6—*Mean Age of Persons with Carcinoma Coming to Autopsy in the Period from 1917 to 1937*

Period	Total		Women		Men	
	Number	Mean Age, Yr	Number	Mean Age, Yr	Number	Mean Age, Yr
1917-1927	132	$56.8 \pm 0.85$	59	$57.9 \pm 1.20$	73*	$56.0 \pm 1.41$
1928-1937	292	$60.3 \pm 0.76$	95	$57.2 \pm 1.48$	197	$61.8 \pm 0.85$
1917-1937	424	$59.2 \pm 0.60$	154	$57.5 \pm 1.10$	270	$60.5 \pm 0.69$

\* One man of "unknown age" was excluded

cantly, but all three of these values were significantly lower than the mean age of men dying with carcinoma in the period from 1928 to 1937. This difference is principally the result of the unusually high age at death of the group with prostatic carcinoma, 27 of the 32 prostatic carcinomas having been observed in the period from 1928 to 1937. If men with carcinoma of the prostate are excluded in calculating the mean age of the male group, the age at death of both men and women in each of the two decades shows no significant deviation from the grand mean of 58.4 years.

TABLE 7—*Mean Age at Death of Persons with Carcinoma at Various Major Sites*

Site	Number	Mean Age, Yr
Prostate	32	$71.5 \pm 1.90$
Liver and bile ducts	26	$62.7 \pm 2.19$
Esophagus	27	$61.1 \pm 1.63$
Rectum and sigmoid	52	$57.8 \pm 1.55$
Stomach	58	$57.7 \pm 1.41$
Lung	43	$55.0 \pm 1.58$
Uterus	29	$52.2 \pm 1.99$

The high age of the prostatic carcinoma group is shown in table 7, which gives the mean age at death of persons with carcinoma at various major sites. With the exception of the uterine carcinoma group, the pulmonary cancer group has the lowest mean age ( $54.95 \pm 1.58$  years). This was significantly lower than the mean ages of the groups with carcinoma of the prostate, liver and bile ducts, and esophagus, respectively, but did not differ significantly from the mean ages of the groups dying with cancer of the stomach, rectum and uterus, respectively.

The analysis up to this point has revealed that there was a shift to a higher level in the age at death of the general autopsy population in the period from 1928 to 1937 and that both males and females participated in this shift. There was no resultant difference in the incidence of carcinoma in either sex, and further, with carcinoma of the prostate excluded, the age at death of persons with carcinoma was essentially the same for both sexes and for each decade of the survey. If it can be shown that the frequency of carcinoma of the lung increases with increasing age, the demonstrated rise in the incidence of this neoplasm in the present survey might be explained on the basis of the general shift in age of the autopsy population to a higher level. That this shift to a higher age level during the second decennial of this survey did not

TABLE 8—*Incidence of Carcinoma of the Lung Among Men—Observed and Expected Values*

Age	1917-1937			1917-1927			1928-1937		
	Men	Number with Carcinoma of Lung	Per- centage	Men	Number with Carcinoma of Lung		Men	Number with Carcinoma of Lung	
					Ob served	Ex pected		Ob served	Ex pected
20-29	189	0	0	69	0	0	120	0	0
30-39	228	2	0.88	103	1	0.9	125	1	1.1
40-49	419	10	2.39	139	2	3.3	280	8	6.7
50-59	413	14	3.39	117	4	4.0	296	10	10.0
60-69	372	9	2.42	100	1	2.4	272	8	6.6
70-79	193	3	1.20	42	0	0.7	151	3	2.3
80-89	51	0	0	13	0	0	38	0	0
90-99	7	0	0	4	0	0	3	0	0
Unknown	15	0	0	8	0	0	7	0	0
Total	1,887	38	2.01	595	8	11.3	1,292	30	26.7

account for the observed increase in the frequency of pulmonary cancers is demonstrated in table 8, which is based on the age distribution of 38 pulmonary carcinomas among men (5 among women are excluded) related to the male autopsy population in each age group. It will be noted that the incidence of carcinoma of the lung rose gradually to its highest value of 3.39 per cent in men 50 to 59 years of age and then fell to 1.20 per cent in men aged 70 to 79, no pulmonary carcinomas were observed in 58 men older than 79 years. From these values obtained from the entire twenty year period of the survey, the number of pulmonary cancers in men was estimated for the period from 1917 to 1927 and for that from 1928 to 1937. The estimated values did not differ significantly from the observed numbers.

In this connection it is of interest to note a somewhat different finding as regards carcinoma of the prostate (table 9). Here the precipitous increase in incidence beginning at the age of 80 to 89 is



striking Prostatic carcinoma was noted in 9 of the 58 men over 79 years, in contrast to cancer of the lung, which did not occur in any of the men comprising this age group This finding confirms the observations of Rich<sup>11</sup> and of Moore,<sup>12</sup> who noted an increasing incidence of prostatic carcinoma with increasing age It is conceivable, therefore, that a further increase in life span might result in a future marked increase in prostatic malignant tumors With regard to the present survey, however, it is apparent that the increased incidence of pulmonary carcinoma was not influenced by the demonstrated increased span of life

4 *No Comparable Increase in Tumors of the Skin*—Frissell and Knox stated that “since external and readily diagnosed cancer has not increased, it would seem probable that the increase in the

TABLE 9—Incidence of Carcinoma of Prostate—Observed and Expected Values

Age	1917 1937			1917 1927			1928 1937		
	Number with Carcinoma			Number with Carcinoma of Prostate			Number with Carcinoma of Prostate		
	of			Ob			Ob		
	Men	Prostate	Percentage	Men	served	Expected	Men	served	Expected
20 29	189	0	0	69	0	0	120	0	0
30 39	228	0	0	103	0	0	125	0	0
40 49	419	1	0.24	139	0	0.3	280	1	0.7
50 59	413	3	0.72	117	1	0.8	296	2	2.1
60 69	372	12	3.22	100	1	3.2	272	11	8.8
70 79	193	7	3.63	42	1	1.5	151	6	5.5
80 89	51	7	13.73	13	1	1.8	38	6	5.2
90-99	7	2	28.57	4	1	1.1	3	1	0.9
Unknown	15	0	0	8	0	0	7	0	0
Total	1,887	32	1.7	593	5	8.7	1,292	27	23.2

incidence of bronchial carcinoma in the last two decades is apparent rather than real” This point has also been made by Fried “It is remarkable that parallel investigations on the incidence of external cancers show no increase” The statement that an increase in tumors of the skin has not occurred is not indisputable Mackee and Cipollaro<sup>13</sup> reported that, “of 76,274 deaths caused by malignant neoplasms in the registration area of the United States in 1921, 2,610 (3.42 per cent) were due to cancer of the mouth Malignancy of the skin was given as the cause of death in 2,433 cases (3.19 per cent) In 1934 the corresponding figures were 134,428 cancer deaths, 5,009 due to cancers of the mouth and 3,315 due to cancers of the

11 Rich, A. R. J. Urol. **33** 215, 1935  
12 Moore, R. A. J. Urol. **33** 224, 1935  
13 Mackee, G. M., and Cipollaro, A. C. Cutaneous Cancer and Precancer, New York, American Journal of Cancer, 1937

skin Thus during a period of thirteen years death from cancer of the buccal cavity and skin showed an increase of 3,280” However, even if there were convincing evidence that the incidence of cutaneous tumors has not increased, there is no logical reason for assuming a corresponding constancy in the incidence of malignant growths of any of the visceral organs In the opinion of Peller,<sup>14</sup> an inverse relationship exists between cancer of the skin and lip and cancer of other sites This, however, has been seriously questioned in a recent report by Conrad and Hill,<sup>15</sup> who found on the average a slight direct association, occupational groups with a relatively high rate of cancer of the skin and lip tending to show also an excess of cancers of other sites In any event, there is no available evidence to indicate a significant relationship between cancer of the skin and lip and carcinoma of the lung

To what extent are conclusions drawn from the numbers of persons coming to autopsy applicable to the general population? This question cannot be answered categorically Although autopsy experience is based on a relatively small proportion of the general population, pathologic diagnoses are admittedly more accurate than clinical ones Whether or not in the problem at hand accuracy in diagnosis is sufficient to compensate for deficiency in size of sample is not known Certain evidence bearing on this problem is, however, worthy of mention

Hirsche<sup>16</sup> studied 1,700 death certificates representing the total mortality from cancer in resident and nonresident citizens of New Haven during the seven year period from 1925 to 1931 It was observed that 803, or 47 per cent, of the deaths occurred in the three main hospitals of New Haven, and that 237, or 14 per cent, of the deaths took place in the New Haven Hospital Thus 1 of every 7 persons whose death certificates bore a diagnosis of cancer in the period of Hirsche's review, died in this hospital Although the number of necropsies performed on this group is not known, the size of the sample is sufficiently large to permit tentative conclusions concerning the population from which the sample was derived

A second study by Hirsche<sup>17</sup> indicates the inaccuracies of cancer death certificates, both as to content and as to total number of deaths from cancer This investigator examined the death certificates and clinical diagnoses of 217 persons dying of malignant disease and examined post mortem in the department of pathology at Yale University

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14 Peller, S Lancet **2** 552, 1936

15 Conrad, K K, and Hill, A B Am J Cancer **36** 83, 1939

16 Hirsche, H F A Study of Cancer Deaths, in The Cancer Problem in New Haven Report of the Cancer Committee of New Haven 1935, appendix C

17 Hirsche, H F A Comparative Study of Post Mortem Diagnoses, in The Cancer Problem in New Haven Report of the Cancer Committee of New Haven, 1935, appendix B

School of Medicine between Jan 1, 1925 and Dec 12, 1933 The diagnosis given in the death certificate tallied with the cause of death as determined at necropsy in 157, or 72 per cent, of these cases In 25 instances, or 12 per cent, of the total, no mention of a malignant tumor was made on the death certificate, and in 35 cases, or 16 per cent, the primary site of the malignant growth as recorded on the death certificate did not correspond with that observed at necropsy It should be emphasized that these discrepancies occurred on certificates which should have been correct in every instance

The greatest errors occurred among cases of primary pulmonary tumor Nineteen such cases were included in the survey, and in 11, or more than half, the clinical diagnosis was incorrect Tuberculosis was clinically diagnosed in 3, and each of the following diagnoses was made in 1 instance carcinoma of the esophagus, carcinoma of the stomach,

TABLE 10—*Comparison of Cancer Mortality in Persons Twenty Years of Age and Older in the State of Connecticut and in the Series Coming to Autopsy at Yale University in the Period from 1918 to 1937*

		State of Connecticut	Yale Autopsies
1918-1927	Deaths in general *	139,496	956
	Deaths from cancer	14,668	133
	Percentage	10.51	13.91
1928-1937	Deaths in general *	149,378	2,012
	Deaths from cancer	20,293	291
	Percentage	13.55	14.46

\* Deaths of persons over 20 years of age

pleurisy, hypernephroma, neoplasm in centrum ovale, lymphosarcoma and heart disease, in 1 instance the condition was not determined The death certificate in 8 of these 19 cases was incorrect The highest correlation between clinical diagnoses and observations at necropsy was found in the cases of gastric carcinoma An incorrect clinical diagnosis had been made in only 2 of 32 such cases, while in 5 the death certificate was in error

On the basis of the foregoing studies it appears, first, that approximately 1 of 7 local patients whose death certificates bear a diagnosis of malignant tumor died at the New Haven Hospital, and, second, that gross inaccuracies occur in both clinical diagnoses and death certificates, carcinoma of the lung being the most frequently misdiagnosed malignant tumor Since the data on the mortality from cancer are characterized by errors both of omission and of commission, as indicated by the death certificates, it is of interest to compare the cancer death rates in the two decennials of the present survey with the corresponding values for the state of Connecticut, these rates being based on the total numbers of

persons over 20 years of age Table 10 indicates a mortality of 13.91 per cent from cancer in the reported material in the years 1917 to 1927 as compared with a mortality of 10.51 per cent in the entire state The next decennial, 1928 to 1937, showed an increase in both series, the value in our own survey being 14.46 per cent, and that in the state, 13.58 per cent If due allowance is made for the greater accuracy of the data recorded from autopsies over those in death certificates, as indicated by Hirsche's study, the discrepancy between the cancer mortality in our autopsy population and that in the state at large would be reduced considerably

In 1920, following an extensive experience with the pathologic aspects of influenza, Winternitz, Wason and McNamara<sup>18</sup> stated that "an increase in primary carcinoma [of the lung] would probably occur later" because of "metaplasia of the bronchial epithelium causing a proliferation of the young cells" The great majority of studies since then have demonstrated the prophesied increase, and although influenza has not been proved definitely to be the factor involved, it nevertheless plays an important role in all discussions of the causes The present report has analyzed certain factors which have frequently been cited to substantiate the belief that this increase is only relative These factors have been shown to have exerted little or no influence on the statistically significant increase in primary carcinoma of the lung found at autopsy in the New Haven Hospital, and it is concluded that the observed increase is real and absolute Indirect evidence appears to justify the additional conclusion that the real increase in primary carcinoma of the lung observed in our autopsy material is characteristic also of the population at large

#### SUMMARY

The 4,156 autopsies performed at Yale University School of Medicine between Sept. 22, 1917 and Dec. 31, 1937 have been reviewed with a view to determining the incidence of carcinoma in general and of carcinoma of the lung in particular The ratio of pulmonary carcinomas to all carcinomas increased from 7.4 per cent during the decade from 1917 to 1927 to 11.0 per cent during that from 1928 to 1937

A method for the calculation of regression equations employing equal numbers of carcinomas as the independent variable instead of equal intervals of time is here introduced The regression equation for carcinoma of the lung calculated on this basis showed a significant positive deviation from zero during the period under review None of the

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<sup>18</sup> Winternitz, M. C., Wason, I. M., and McNamara, F. O. *The Pathology of Influenza*, New Haven, Conn., Yale University Press, 1920

regression equations calculated in a similar manner for carcinoma of the stomach, liver and bile ducts, prostate, intestine, female genitalia, esophagus and rectum showed a significant deviation from the horizontal

The factors which might have been responsible for the observed increase in the incidence of primary carcinoma of the lung were analyzed. It was found that the general autopsy population of persons over 20 shifted to a higher age level during the period from 1928 to 1937 as compared with the previous decade. This shift, however, did not account for the observed increase in the incidence of primary pulmonary tumors.

It was concluded that the increase in pulmonary cancers observed in our autopsy material was a real and absolute increase. Indirect evidence appeared to indicate that this conclusion was also applicable to the general population.

# DISSECTING ANEURYSM OF THE AORTA IN EXPERIMENTAL ATHEROSCLEROSIS

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There is a growing interest in the study of dissecting aneurysm of the aorta. This has been stimulated mainly by the successful crystallization of knowledge of the clinical features of the disease. The condition is being more frequently recognized at the bedside rather than at the autopsy table. The incidence of the condition appears to be increasing. Thus in a group of 3,206 necropsies in the Boston City Hospital during the years from 1927 to 1933, inclusive, dissecting aneurysms were found in 1 case out of 320,<sup>1</sup> while in a group of 2,202 necropsies from 1935 to 1937, inclusive, the proportion was 1 to 200.<sup>2</sup> During the same period (1935-1937) syphilitic aneurysms of the aorta occurred in 1 case out of 160. A comparison of the frequency ratios of the two conditions suggests that, with the expected future decrease of syphilis and increase of vascular degenerative disease, dissecting aneurysm may eventually become more frequent than syphilitic aneurysm. Thus dissecting aneurysm is more than a clinical curiosity.

In the genesis of dissecting aneurysm medionecrosis aortae is the usual predisposing factor, with hypertension the common activating agency. Atherosclerosis is responsible for a certain percentage of cases, and rheumatic aortitis is said to be a possible cause. Physical or mental disturbances may be contributing agents in the actual rupture, probably because of their influence on hypertension, and mechanical injury (external or internal trauma) has been reported as an exciting element.

## EXPERIMENTAL DISSECTING ANEURYSM

Before discussing experimental lesions it is necessary to consider the possibility of the occurrence of spontaneous dissecting aneurysm in the rabbit. Personal experience and special inquiries in laboratories, veterinary schools and zoos have failed to reveal cases. Liebig<sup>3</sup> made a

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1 Weiss, S. *M Clin North America* **18** 1117, 1935

2 Weiss, S. *New England J Med* **218** 512, 1938

3 Liebig, H. *Arch f exper Path u Pharmacol* **159** 359, 1931

definite statement that spontaneous aneurysm of the aorta of the rabbit is unknown

Bennecke,<sup>4</sup> in a study of lesions of vessels following the use of poisons, recorded his findings in 400 rabbits that were not subjected to experimentation but died spontaneously or from intercurrent infections. He pictured without description an aortic malformation observed in a rabbit which may have been a short dissecting aneurysm, and also an irregular dilatation of the aorta of another of these animals. In the literature on the subject which he discussed there was no reference to similar lesions, and no publications since that time have recorded such changes in normal rabbits.

#### MEDIAL NECROSIS IN THE RABBIT

The media of the aorta of the rabbit is not infrequently the seat of minute lesions. These are sharply localized and consist of degenerative and necrotic processes with a strong tendency toward calcification. Miles and Johnstone<sup>5</sup> and Levin and Larkin<sup>6</sup> described lesions, practically all of which were minute, in 35 to 52 per cent of normal rabbits. These lesions were limited, almost without exception, to the media or were of medial origin.

#### EPINEPHRINE-INDUCED NECROSIS IN THE MEDIA OF THE RABBIT AORTA

The discovery of medial lesions in the aorta of the normal rabbit was used to discountenance claims that aortic lesions following injections of epinephrine hydrochloride were due to that drug. Lesions produced in the aorta of the rabbit by the vasopressor effects of epinephrine, however, are more extensive than the minor processes found in normal rabbits. Josue<sup>7</sup> first reported the effects of injections of epinephrine in 1903. The resemblance of these lesions to those of medionecrosis aortae in the human aorta is close. As would be expected, some dissecting aneurysms occurred in these experimental animals.

Fisher<sup>8</sup> described a dissecting aneurysm extending from the arch of the aorta to the level of the renal arteries in an animal in which paralysis of both hindlegs developed following the twenty-third injection of epinephrine. He emphasized the fact that the lesions of the aorta following repeated injections of epinephrine are a "primary necrosis of the muscular and elastic layers of the media" and that these lesions have little in common with the changes observed in atherosclerosis of the human aorta.

4 Bennecke, A. *Virchows Arch f path Anat* **191** 208, 1908.

5 Miles, A. B., and Johnstone, O. P. *J A M A* **49** 1173, 1907.

6 Levin, I., and Larkin, J. H. *J Exper Med* **13** 24, 1911.

7 Josue, O. *Compt rend Soc de biol* **55** 1374, 1903.

8 Fisher, B. *Deutsche med Wchnsch* **31** 1713, 1905.

Erb<sup>9</sup> described among a large group of experimental animals a young rabbit, weighing 1.3 Kg, which received over a period of about two months thirty-one injections of 0.1 to 0.3 cc of a 1:1,000 solution of epinephrine hydrochloride. A few minutes after the last injection flaccid paralysis of the hindlegs and severe dyspnea developed. The animal died six hours later. Necropsy revealed hemoperitoneum, with clots, and retroperitoneal hemorrhage about the aorta. Below the renal arteries the aorta was collapsed and white. There was serosanguinous fluid in both pleural cavities as well as hemorrhage into the tissues about the thoracic portion of the aorta. There was a dissecting aneurysm originating in the lower part of the arch and extending to the level of the renal artery. This started between the intima and the media and continued into the media and adventitia.

In Kulbs's<sup>10</sup> series 1 rabbit received thirty-one injections of massive doses of epinephrine in sixty-two days. Death acutely followed the last injection. Necropsy revealed hemorrhages into both pleural cavities. The mediastinal tissues were infiltrated with blood. There was an intimal tear, the location of which was not mentioned. The dissection extended downward to the iliac junction. On microscopic examination there was separation of the intima and media at the site of the original rupture. Elsewhere the adventitia was elevated by hemorrhagic dissection.

Among Ziegler's<sup>11</sup> animals a dissecting aneurysm developed in 1 rabbit. An accidental fall from a height of 1.5 meters precipitated the rabbit's death. This animal, 8 weeks old, had received seven injections of 0.15 cc of a 1:1,000 solution of epinephrine hydrochloride. At postmortem examination there was hemorrhagic infiltration of the periaortic tissues with a diffuse hematoma of the aortic wall. There was an intimal tear through which blood penetrated between the intima and the media. Below this the layers of the diseased media were separated, and the adventitia had ruptured at the level of the diaphragm.

Kaiserling<sup>12</sup> reported 1 animal in his series with dissecting aneurysm. This rabbit received thirty-eight doses of a 1:1,000 solution of epinephrine hydrochloride, varying from 0.1 to 0.7 cc, over a period of forty-four days. Several hours after the last dose, 0.7 cc, the animal showed sudden paralysis of the hindlegs, dyspnea and rapid pulse. It became apathetic and was found dead the next morning. Necropsy disclosed two tears in the aortic intima. One, 2 cm long, was in the thoracic aorta with separation and rupture of the adventitia. The second small tear was located immediately below the diaphragm. Here the dissection had

9 Erb, W. Arch f exper Path u Pharmacol **53** 173, 1905

10 Kulbs. Arch f exper Path u Pharmacol **53** 140, 1905

11 Ziegler K. Beitr z path Anat u z allg Path **38** 229, 1905

12 Kaiserling, K. Klin Wchnschr **44** 29, 1907



torn an artery. It is of interest that histologic examination failed to reveal any localized lesion of the aortic media.

Schirokogoroff<sup>13</sup> reported dissecting aneurysms in 2 rabbits of an epinephrine series. A rabbit 3½ months old died fifteen hours after the last injection. The animal had received seventeen injections of 0.15 cc of a 1:1,000 solution of epinephrine hydrochloride. The record of the postmortem examination lacks details, but it is stated that a dissecting aneurysm was present and that there was necrosis of the muscle and elastic fibers of the aortic media. The second rabbit had received forty injections of 0.15 cc of a 1:1,000 solution of epinephrine in three months and four days. In the record of the postmortem observations there is mention merely of the presence of a dissecting aneurysm and necrosis of the muscle layer of the media.

These reports of aortic dissecting aneurysm following injections of epinephrine are in large part lacking in detail. The aneurysms are casually recorded as interesting observations in the course of experiments dealing primarily with the general effects of the drug. The thickness of the human aortic media favors the dissecting apart of its layers by the blood as it spreads. The thin media in the aorta of the rabbit does not lend itself so readily to separation of the fibers, so that in most cases the dissection tends ultimately to occur between the media and the adventitia.

#### LESIONS INDUCED IN THE AORTA OF THE RABBIT BY VITAMIN D

The feeding to rabbits of massive doses of viosterol or of other preparations of vitamin D gives rise to medial necrosis with a strong tendency toward calcification. Dissecting aneurysm of the aorta was not reported as occurring in these animals. The lacking element in these experiments was probably the vasopressor factor.

#### ATHEROSCLEROTIC (INTIMAL) LESIONS IN NORMAL RABBITS

It is apparent that the lesions found in normal rabbits and those produced by epinephrine or by vitamin D are limited to, or arise from, the media.

Clarkson and Newburgh<sup>14</sup> cited records of 2,947 rabbits reported by several workers in experimental medicine with ten possible examples (0.34 per cent) of spontaneous sclerosis of the aorta which might be of the atherosclerotic type. Atherosclerosis can be produced, however, in 100 per cent of rabbits by feeding them adequate amounts of cholesterol, and the lesions will cover much of the surface of the aorta.

<sup>13</sup> Schirokogoroff, J. J. *Virchows Arch f path Anat* **191**: 482, 1908.

<sup>14</sup> Clarkson, J., and Newburgh, L. H. *Arch Int Med* **31**: 653, 1923.

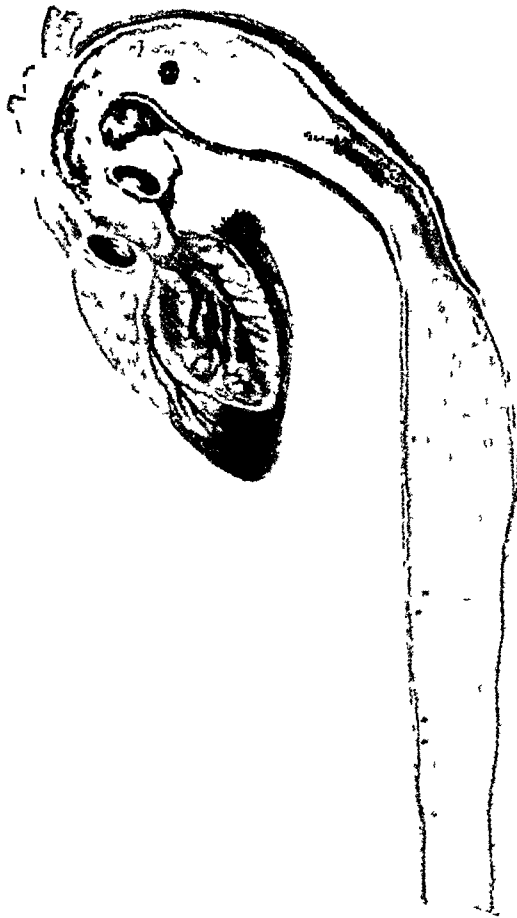


Fig 1—Dissecting aneurysm of the aorta of a rabbit (see text for a full description)



in contrast to the relatively minute lesions described so rarely in normal rabbits. There can be no confusion, therefore, between the rare processes found in normal animals and the constant results of adequate cholesterol feeding.

#### REPORT OF A CASE

A male rabbit had been fed a total of 60 Gm of cholesterol in solution in sunflower seed oil by catheter. There were 110 feedings from Oct 22, 1934 to May 7, 1935. The weight of the rabbit increased during that period from 1,780 to 2,880 Gm. Feeding had progressively increased from 0.3 Gm of cholesterol daily six times a week to 0.9 Gm in January 1935. Because of an epidemic among the rabbits, feeding was stopped Jan 26, 1935, it was resumed March 8 (0.3 Gm) and continued until May 7.

The animal was allowed to live after cessation of the cholesterol feeding. During the three years intervening before its death it was kept on a diet of rabbit chow<sup>14a</sup>. When last seen alive, April 12, 1938, the animal was apparently well. Its weight had been standardized at about 3,400 Gm for some time before death.

On the morning of April 13, 1938 the animal was found dead in its cage. At postmortem examination the right pleural cavity contained currant jelly clots and a small amount of fluid blood. The left pleural cavity contained a small amount of clot and fluid blood. There was hemorrhagic infiltration of the periaortic tissues downward to the level of the diaphragm. There was hemorrhagic infiltration of the tissues about the esophagus up to the base of the skull. Rupture into the pleural cavity on the right had occurred through a slitlike opening in the pleura just above the diaphragm. The point of rupture into the left pleural cavity could not be found. The aorta was tortuous, the wall was thickened and opaque, and there was dilatation of the arch and upper thoracic portion. When the vessel was laid open, an oval ulceration 0.5 by 0.3 cm, was seen in the anterior wall, just below the junction of descending arch and thoracic portion. This was lined by a granular red membrane. The layer of blood, located between the deep layers of the vessel wall, extended upward along the aorta to the level of the orifice of the left subclavian artery and downward almost to the diaphragm (fig 1). It varied in thickness up to 0.9 cm. Calcification of the wall was continuous in the lower thoracic region, producing a waistlike effect. The abdominal portion of the vessel was dilated, but the wall was less markedly thickened than in the thoracic portion. The aortic intima was thickened, nodular and colored brownish yellow, with yellow predominating in the nodules. The nodular and diffuse thickening of the intima was continuous down to the midabdominal region. Below this level were scattered yellow nodules. The vessels of the arch showed marked thickening of their walls with great narrowing of their lumens, that of the right carotid artery being almost occluded. The walls of the carotid arteries showed small patches of yellow opaque thickening to within 1 cm of the base of the skull.

The heart was hypertrophied, the cavities were empty. The left and right coronary arteries showed thickened walls and narrowed lumens.

The right lung was compressed and airless except over a narrow region along the anterior edge. The left lung had compression atelectasis over the back of the lower lobe but was otherwise normal. The bronchi and trachea were normal. The liver was of normal size and a gray-red color, with focal regions of irregular lobulation and scarring. The gallbladder contained an estimated 3 cc of bluish

<sup>14a</sup> This chow is a standard stock feed containing all necessary food substances but no animal cholesterol as such.

black bile. The mucosa was natural. In the middle portion of the spleen was a firm subcapsular nodule, 0.7 cm in greatest diameter, which on section was opaque yellowish white. Apart from this nodule the organ was normal.

The left kidney was of normal size, the right, smaller. The capsules peeled with little difficulty. The surface of the right kidney showed a series of depressed scars, beneath which, on section, the cortex was thinned. The surface of the left kidney was smooth. The cut surfaces showed bilaterally a series of opaque yellow streaks running from the intermediate zone toward the apexes of the pyramids.

The adrenals, though of normal size, were softer than normal. On section there was a suggestion of thickening of the cortex. The testicles were of normal appearance, and the thyroid, the brain and cerebral arteries and the gastrointestinal tract were likewise normal. There was no gross evidence of cholesterol deposits in the eyes or in the skin.

*Microscopic Examination*—(a) *Aorta*. Near the site of the atheromatous ulcer the aortic wall showed, together with thinning and hyaline degeneration of the media, a markedly thickened intima made up largely of hyaline connective tissue. In the base of the ulcer, at one side the intima was almost wholly replaced by a richly cellular tissue, made up of young fibroblasts paralleling one another, with their long diameters at right angles to the media or remaining deep fragments of the intima. This young granulation tissue was diffusely infiltrated with lymphoid and plasma cells, macrophages and a few polymorphonuclear leukocytes. In places the continuity of the intima was broken and the granulation tissue was based on the hyaline remnants of the media. In the midregion of the ulcer was a hyaline fibrinous thrombus undergoing organization which in places where the media was absent rested directly on the adventitia. The general picture was that of deep necrosis of the thickened intima and in part of the media, followed by thrombosis and progressing organization of the clot (fig 2 A).

Alongside the ulcer a hemorrhage into the wall could be seen at one point, separating the layers of the media (fig 2 B), but for the most part the clot lay between the media and the adventitia as it spread up and down the aorta.

The aortic wall in general owed its thickening largely to an intimal process. It was made up in great part of a connective tissue varying in density from that of moderately cellular collagenous tissue to dense, relatively acellular, hyaline connective tissue (fig 3 A). The surface layer was loose textured and showed infiltration with lipid foam cells and lymphocytes. In sections stained for fat the middle and deep layers of the intimal fibrous tissue contained abundant isotropic granular lipid.

(b) *Heart*. The muscle fibers were hypertrophied. In the main coronary arteries the intima was thickened by hyaline fibrosis, and the lumens were narrowed. In some of the muscular branches the narrowing of the lumens was marked (fig 3 B), and one smaller branch showed complete occlusion. At the summit of some of the muscle folds, beneath the endocardium the muscle fibers were shrunken, distorted and in part replaced by relatively acellular connective tissue (fig 4 A).

(c) *Carotid Arteries*. The intima of the right carotid artery (fig 4 B) was thickened by a growth of connective tissue, which had narrowed the lumen to an angular slitlike opening. In a deeper crescentic fold a hyaline thrombus was undergoing organization, and the inner layer of the intima was made up of young fibroblastic tissue infiltrated with lymphoid cells and monocytes. In the other folds the endothelial layer lay on a relatively loose-textured connective tissue, which made up much of the inner layer of the intima. Near the media there was an encircling layer of dense hyaline connective tissue, thrown into fluted folds, internal

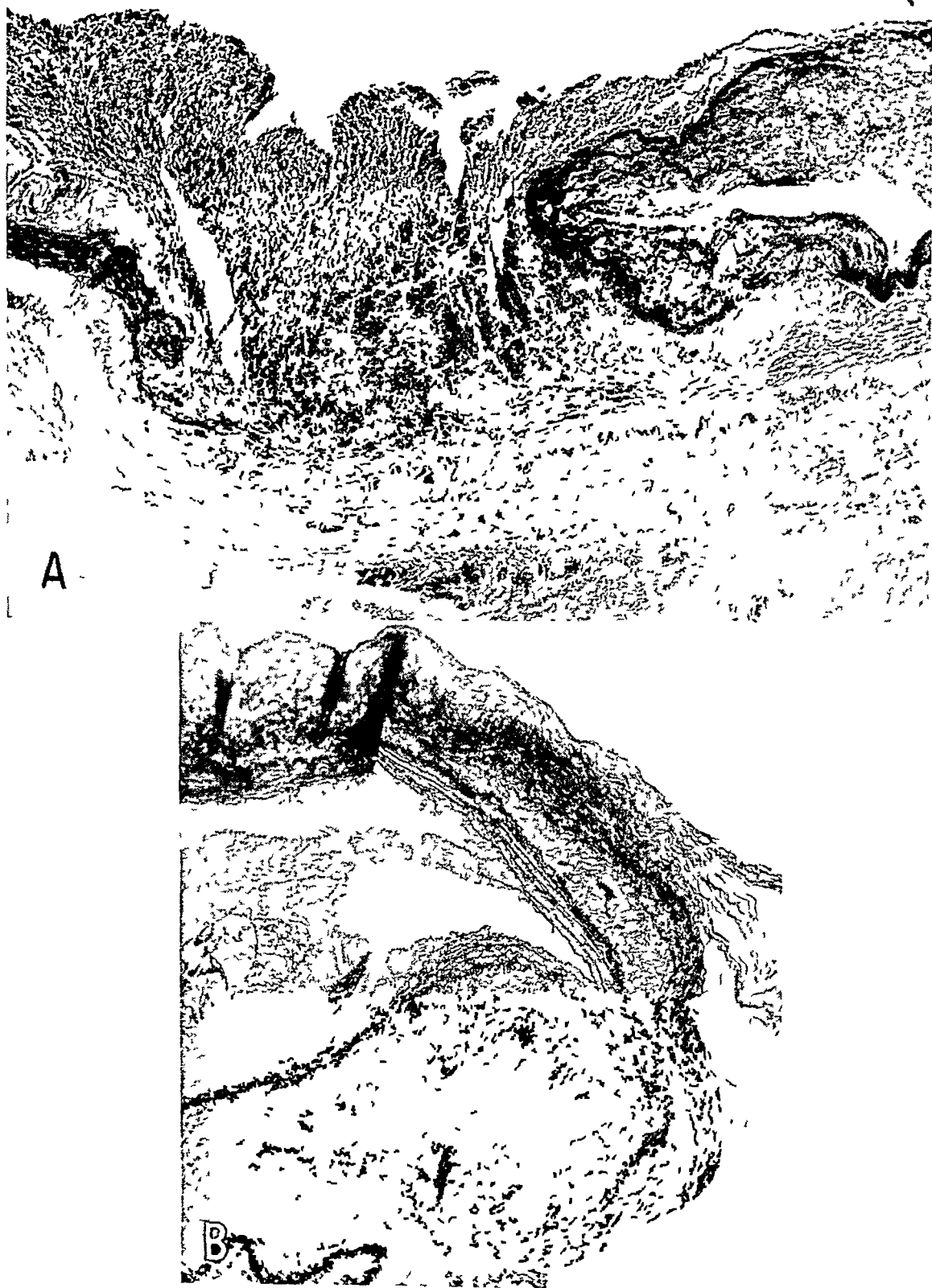


Fig 2—*A*, atheromatous ulcer with thrombosis (see text) *B*, blood dissecting apart the layers of the aortic media near the ulcer



Fig 3—*A*, aortic atherosclerosis The thickening of the intima is largely due to fibrosis and hyalinization of the fibrous tissue Below is a mass of blood outside the media, actually separating the media from the adventitia

*B*, fibrous narrowing of the lumen of a coronary branch—an old healed lesion

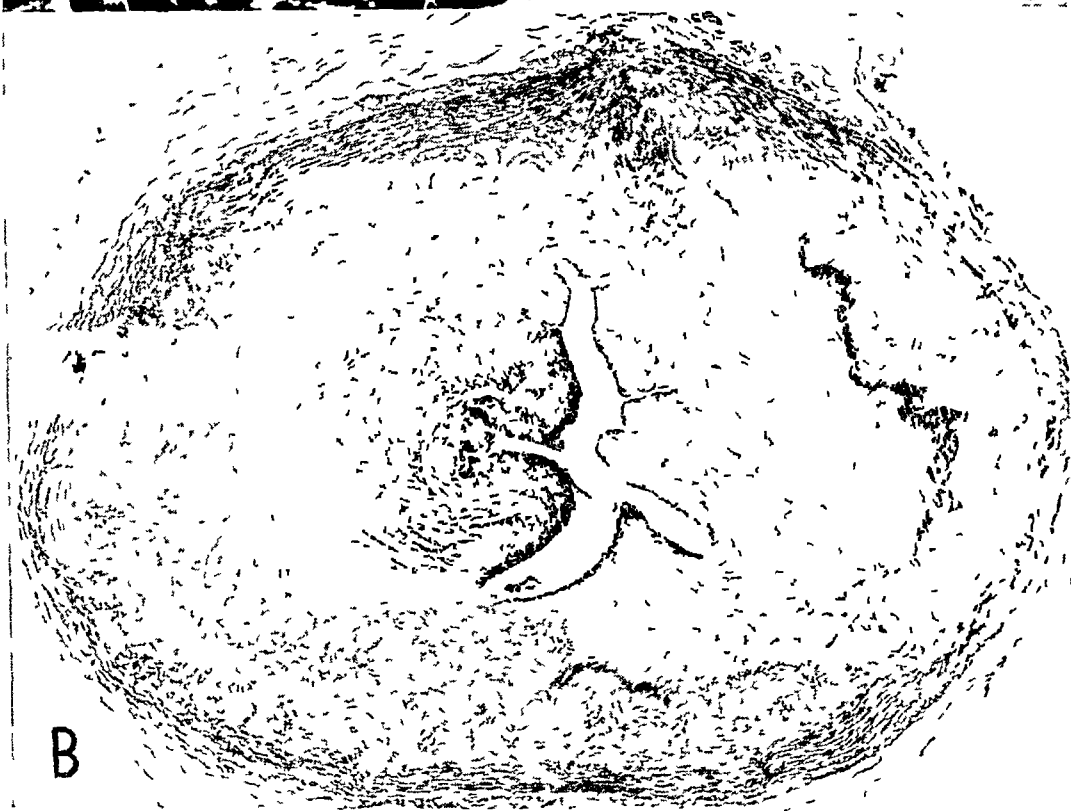
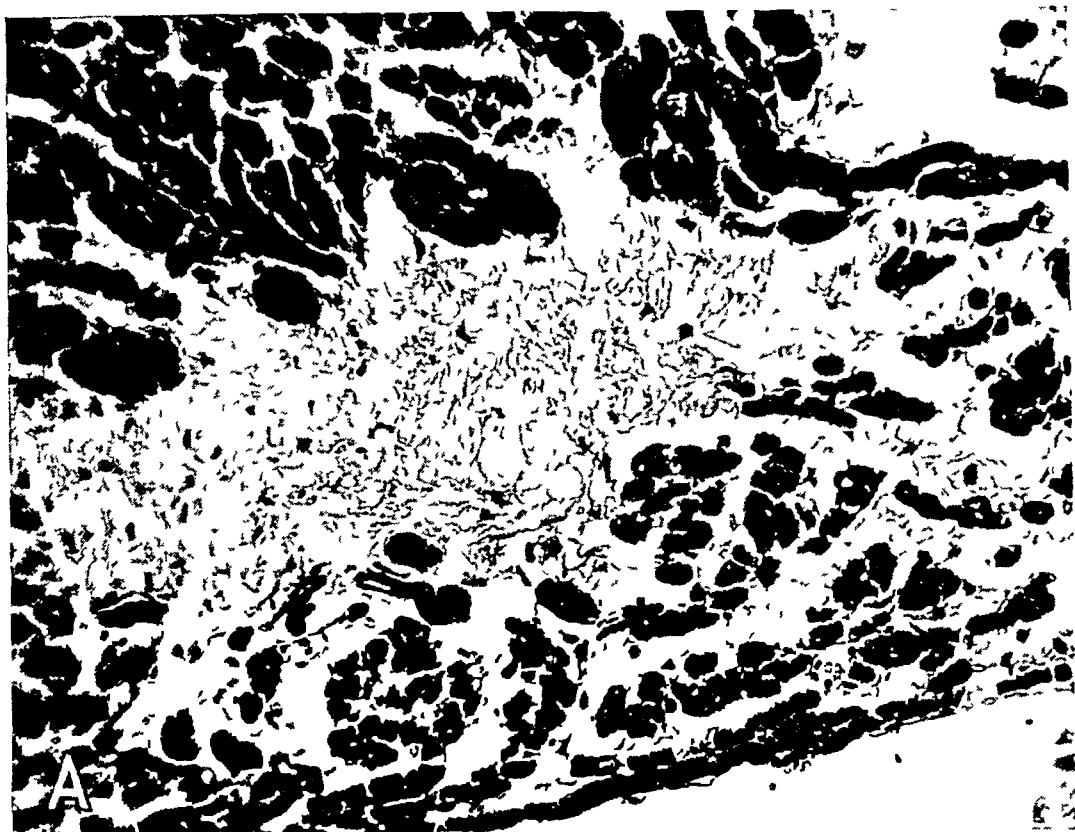


Fig 4—*A*, old repaired atrophy and necrosis of cardiac muscle fibers (chronic vascular myocarditis) Compare the size of the fibers to the right and below with those to the left and above *B*, section of the right carotid artery about 0.5 cm from its origin (see text)



to which in places was an acellular material staining pink with eosin (In frozen sections stained for fat this material and the dense hyaline layer surrounding it were rich in sudan-staining granular lipoid Under the polariscope some anisotropic crystals were scattered through the fatty material) The media showed irregular thinning, and in places only a few fibers remained At points where vasa vasorum were penetrating through the media there was some infiltration with lymphoid cells, and the adventitia about these regions showed lipoid cell infiltration In frozen sections stained with sudan IV there were lipoid cells scattered in places in the media Sections stained by Verhoeff's or Weigert's elastic stains disclosed irregularity, fragmentation and disappearance of the elastica in the media In the fluted hyaline layer there was an irregular production of elastic tissue in the form of series of fine fibrils, with a heavier elastic band along the fluted edge This inner elastic lamina could be followed for some distance as an intact membrane and then was seen to become granular and disappear in the fatty detritus in this region

The left carotid and the subclavian arteries were the seat of similar lesions, the right subclavian artery showing a lumen so narrow that at one level the intimal folds were almost in contact

(d) Liver For the most part the organ was normal In the regions of irregular lobulation there was an increase of periportal connective tissue outlining the lobules, many of which were distorted There was moderate infiltration of the periportal tissue by lymphocytes

(e) Spleen The splenic tumor was poorly encapsulated and was made up of oat-shaped cells, which tended to occur in whorls, as in a meningioma Mitoses were present Lipoid-containing cells were few Otherwise the spleen was not remarkable

(f) Kidneys The arteries of the right kidney showed high grade fibrous sclerosis with narrowing of the lumens, which was extreme in places In the regions of thinning of the cortex there was diffuse fibrosis with lymphoid cell infiltration Glomeruli were found in all stages from thickening of the capsule, through adhesion of the tufts, to complete hyaline scarring Alongside these regions the cells lining convoluted tubules were swollen and granular, with fine granules which took the fat stain Much of the renal structure away from the regions of fibrosis was normal, and the tubular epithelium contained no fat granules The yellow streaks seen grossly in the pyramids were made up of collections of cholesterol crystals embedded in fibrous tissue

The left kidney was essentially normal save for masses of cholesterol crystals shut off in dense connective tissue in streaks in the pyramids

(g) Adrenals The cortical layer was thickened and rich in cholesterol Lying in the zona reticularis were many small collections of cells, which took a purple stain with Nile blue sulfate Similar groups of cells were present in lymph vessels in the medulla The contents of these cells showed little or no anisotropism, a rare crystal being present in an occasional group, in contrast to the cortical cells, which were rich in cholesterol esters

The anatomic diagnoses were exsanguination, bilateral hemothorax, dissecting aneurysm of the aorta, atheromatous ulcer of the aorta, advanced atherosclerosis of the aorta with calcification and dilatation of the arch and the thoracic portion, atherosclerosis of the carotid, of the subclavian and of the coronary arteries, hypertrophy of the heart with focal chronic vascular myocarditis, chronic vascular nephritis on the

right, embedded cholesterol crystals in renal pyramids bilaterally, focal subacute and chronic interstitial hepatitis, fibrosarcoma of the spleen

#### HUMAN AND EXPERIMENTAL ATHEROSCLEROSIS

Anitschkow,<sup>15</sup> in his review of experimental atherosclerosis, records with respect to atherosclerotic lesions in the rabbit the following variations from human processes

*The principal differences between experimental cholesterol atherosclerosis in rabbits and human atherosclerosis are as follows* (1) In experimental atherosclerosis no fatty infiltration of the elastic fibers or lamellae is ever observed, as is often the case in human patients. In rabbits, the lipid substances generally accumulate only in the ground substance of the arterial wall and in the cellular elements of the newly formed intima. (2) In material derived from the experimental animals one finds much larger quantities of lipoidal cells than is generally the case in human material of a corresponding nature. It is true though that in human material the quantity of lipoidal cells found in the newly formed intima is subject to great variations, and in some cases it is very considerable. (3) In cholesterolized rabbits neither the hyaline swelling of the fibrous atherosclerotic plaques nor the development of fibrous elements in general is as pronounced as in human atherosclerosis. (4) In rabbits no ulcers are ever observed in the atherosclerotic plaques, whereas in human atherosclerosis such ulcers are quite frequent, they are sometimes covered with thrombotic deposits. (5) The distributions of the atherosclerotic changes in the arteries is somewhat different in rabbits, as compared with human atherosclerosis, although the arteries affected are generally the same. The cerebral arteries however—to mention one exception—are never affected in rabbits.

It is evident from the material presented in this case that most of the differences which Anitschkow cites<sup>1</sup> resulted from the comparison of early experimental lesions with late human lesions. The study of a large series of rabbits which were fed cholesterol and *permitted to live for years after cholesterol feeding ceased* has demonstrated that when late rabbit lesions are compared with standard (late) human atherosclerotic lesions the differences are minor. Thus, with reference to Anitschkow's claimed differences

1 Fatty degeneration of the elastica is a late human lesion, appearing as the process includes the media. Similarly, in late experimental lesions fatty degeneration occurs in the elastica.

2 Figures 3 and 4 B indicate that in late experimental lesions lipid cells are at least no more abundant than in human lesions.

3 These illustrations also establish that in the experimental lesions hyaline changes in the connective tissue and the fibrosis in general correspond to those found in late human lesions.

4 The atheromatous ulcer in this case negatives claimed difference 4.

<sup>15</sup> Anitschkow, N., in Cowdry, E. V. *Arteriosclerosis. A Survey of the Problem*, New York, The Macmillan Company, 1933, pp. 305-306.

5 There are differences in the localization of the aortic lesions in the experimental and the human disease. In man the ascending portion of the aorta resists the production of advanced atherosclerotic lesions. This region is a favorite site for the early deposit of lipid cells. Crops of pinhead-sized lesions, due to invasion of the subendothelial layer of the intima by these cells, are frequently met with. These may spread in fanlike extensions over the intima but remain superficial. The cholesterol in lesions of this portion of the aorta is removed by fibroblastic cells, as one of us (T. L.) has demonstrated<sup>16</sup>. The lesions lose their orange color, become pale yellow, then gray, and finally disappear. The cholesterol provokes a growth of fibroblastic tissue in these lesions, but this tissue does not form collagen, so that scarring does not follow. The mechanism referred to also removes cholesterol deposits from the aorta and other vessels in the young. It is efficient, even into old age, in the ascending portion of the aorta, though it shows little or no activity in other parts of the vessel.

In the rabbit there is no resistance of the ascending portion of the aorta to the production of atherosclerosis, or else the large doses of cholesterol in the diet overwhelm the mechanism of resistance. As a result, the lesions extend continuously from the ring over the arch and thoracic portion of the aorta. In the late stages dilatation occurs throughout this region and calcification, often continuous, is usual.

The resemblance of this continuous atherosclerotic process, with its dilatation and calcification, to the atherosclerotic lesions associated with human syphilitic aortitis is close. In syphilitic aortitis the resistance of the ascending aorta to atherosclerosis is abolished, and a secondary atherosclerotic process is found—continuous from the ring and over the arch into the thoracic portion of the aorta. This is accompanied by dilatation and calcification, which is frequently continuous.

Human cerebral arteriosclerosis is usually a very late manifestation of the disease, associated with senility. No experimental rabbits have been permitted to live long enough to correspond in relative age to the senile human being with cerebral arteriosclerosis.

#### COMMENT

In this paper is reported the first atheromatous ulcer of record in an experimental animal and the first dissecting aneurysm in a rabbit fed cholesterol.

In the literature on the subject of dissecting aneurysms in rabbits under experimental conditions it is made clear that this lesion most frequently follows necrosis of the aortic media. In the group of animals which received repeated doses of epinephrine severe paroxysmal arterial

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16 Leary, T. Arch. Path. **21** 419, 1936.

hypertension played the primary role in the causation of the dissecting aneurysm. It is probable that the necrotic lesions of the media resulted from the damaging effect of the hypertension. This type of pathogenesis corresponds to that often present in human subjects in whom there is an association of severe hypertension with medionecrosis but in whom the intima is normal. The significance of hypertension as a factor in the actual rupture is suggested by the failure of the aortas of rabbits fed excessive doses of vitamin D to exhibit dissecting aneurysms, though the medial damage was severe. That hypertension was the factor lacking in these animals is a reasonable conclusion.

In the case reported here no measurements of blood pressure were made. However, the hypertrophy of the heart, manifest both grossly and microscopically, the great narrowing of the lumens of the vessels arising from the aortic arch and the presence of chronic vascular nephritis in one kidney make it probable that hypertension was present in the animal.

The primary factor in this case was a diffuse atherosclerotic process with an atheromatous ulcer, induced by cholesterol. Atherosclerosis is the agency responsible for a small number of dissecting aneurysms in man. Rupture may occur in relation to an atherocheuma (atheromatous abscess, so called) or to an atheromatous ulcer which has resulted from the rupture of an atherocheuma. The two types of genesis of dissecting aneurysm in the rabbit appear to be identical with the mechanisms observed in man.

#### SUMMARY

A dissecting aneurysm of the aorta is reported arising in an atheromatous ulcer in a cholesterol-fed rabbit which was allowed to live for three years after cholesterol feeding was stopped.

The relation of atherosclerosis in man to experimental atherosclerosis in the rabbit is discussed. The necessity of comparing standard (late) human atherosclerotic lesions with late atherosclerotic lesions in the rabbit is stressed.

The causation of dissecting aneurysm appears to be identical in man and in the experimental rabbit. In both the important causative factors are medionecrosis or atherosclerosis, with hypertension.

# BEHAVIOR OF TUBERCLE BACILLI FOLLOWING THEIR INTRAVENOUS INJECTION INTO A RESISTANT ANIMAL (RAT)

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Rats have been shown to be highly resistant to infection with tubercle bacilli, in those cases in which tuberculosis develops, it is usually the Yeisin type, characterized by enormous numbers of bacilli together with proliferation of epithelioid cells, and absence of caseation

This study was undertaken to determine the fate of tubercle bacilli when they are injected intravenously into an animal, namely, the rat which is regarded as highly resistant to them. The recent work of Hehre and Freund suggests that so far as the lungs are concerned there is an initial reduction between about the second and tenth weeks though the rats which died six months or more after injection showed large numbers of the bacilli in their lungs. The figures published by these authors were derived from counts on sections of lung, liver and spleen, each represents the number of organisms seen in 100 microscopic fields. This technic was adopted in the present investigation, and, in addition, the number of colonies which grew from 1 mg of each of these organs was determined by cultural methods

Albino rats about 6 months old and weighing approximately 150 Gm were used. An injection of 1 mg of living tubercle bacilli suspended in 0.1 cc of saline solution was made into the femoral vein under ether anesthesia. The Ravenel strain, isolated more than thirty years ago by Dr. Mazyck Ravenel, was used, this strain is of the bovine type and is highly virulent, one intravenous injection of 0.00001 mg being sufficient to kill a rabbit in three to six months, with extensive tuberculosis. In these respects the experiment was identical with that of Hehre and Freund<sup>1</sup>

The rats were subsequently killed at various intervals. The chest and abdomen were opened under sterile conditions, and specimens of tissue were removed from the center of the lower lobe of the right lung, the center of the right lobe of the liver and the middle of the spleen. Each of these specimens was then treated as follows

1. A part was fixed in solution of formaldehyde U. S. P. diluted 1:10, for twenty-four hours, and was stained by the Ziehl-Neelsen method for tubercle bacilli and also with hematoxylin and eosin. An attempt was made to obtain a rough estimate of the volume of tissue which was examined when the number of

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1. Hehre, E., and Freund, J. Arch. Path. **27**: 289, 1939

organisms in 100 microscopic fields was counted. All sections were cut at 6 microns, and the diameter of the microscopic field was found to be 0.15 mm, hence the volume of tissue in 100 fields was 0.0106 cu mm. By multiplying the number of bacilli seen in 100 fields by 100, the approximate number of visible bacilli in 1 cu mm was obtained.

2 Two hundred to three hundred milligrams of tissue was weighed to the nearest milligram. It was crushed in a mortar, and fifteenth-normal sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) was added in the proportion of 1 cc to 100 mg of tissue. After further crushing, 0.2 cc of this mixture was diluted ten times with the phosphate solution, and 0.2 cc of the 1:10 dilution was further diluted ten times. Two test tubes containing Petragram's medium were inoculated with 0.1 cc of the 1:10 dilution, 1 e, with 1 mg of tissue, and a further two test tubes with the 1:100 dilution, 1 e, with 0.1 mg of tissue. After incubation

TABLE 1—*Bovine Tubercle Bacilli (Ravenal Strain)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
100	½ hour	120	0	0	800	200	80
101	½ hour	240	10	0	400	200	50
104	1 week	25	125	85	300	4,000	1,500
105	1 week	0	215	120	1,000	3,000	5,000
80	2 weeks	700	650	900	7,000	15,000	10,000
81	2 weeks	0	0	0	70	100	100
106	3 weeks	530	15	20	150	40	400
107	3 weeks	540	295	320	1,000	400	1,000
82	4 weeks	250	150	65	3,000	1,000	1,500
83	4 weeks	225	65	185	1,500	1,500	1,000
84	6 weeks	430	105	45	3,000	400	600
85	6 weeks	370	35	135	5,000	300	300
88	2 months	1,760	75	130	∞ (15,000)*	1,500	2,000
89	2 months	2,630	55	315	∞ (9,000)	1,000	2,000
90	6 months	345	15	10	4,000	1	200
91	6 months	∞	65	40	∞ (15,000)	20	400
92	6 months	105	80	15	∞ (10,000)	50	500
93	6 months	∞	0	45	∞ (20,000)	75	1,000
94	6 months	180	0	5	∞ (12,000)	750	750

\* The symbol ∞ is used to indicate that the number of colonies was too large to count.

of the cultures at 37 C for about three weeks colonies began to appear, and these were counted from time to time until the end of the sixth week. A mean figure was then assessed from the numbers of colonies in the four test tubes and was expressed in terms of the number of colonies growing in one of the tubes containing the 1:10 dilution. It was approximately the number of colonies in 1 mg of tissue.

Three rats died during the course of the experiment. The results obtained on the remaining 19 rats are shown in table 1.

## RESULTS

*Lungs*—During the first two months the numbers of bacilli indicated a tendency to multiply, and in all the rats killed two to six months after injection cultures showed the numbers were considerably increased. It was impossible to estimate a mean figure for the number of bacilli in 100 fields in the animals killed at six months because in 2 of the 5 rats the bacilli were too numerous to count. Also, in the rats killed at six months the number of colonies (stated in parenthesis) was assessed from the findings in the 1:100 dilutions, as all the tubes containing the 1:10 dilution showed an infinite number of colonies.

*Liver and Spleen*—Table 1 shows that there was an initial multiplication of bacilli in these organs, this increase was not maintained, a much smaller number being found at the end of six months

The large number of colonies and bacilli found in rat 80 as compared with the other rats killed after the shorter intervals cannot be easily explained. The animal appeared to be healthy, and at autopsy no intercurrent disease was found. It is evidence that occasionally a rat shows little resistance to the multiplication of tubercle bacilli after intravenous inoculation.

In order to investigate more fully the behavior of tubercle bacilli during the first few weeks, a further series of rats was given intravenous injections of a small quantity of tubercle bacilli, namely, 0.01 mg. The Ravenel strain was

TABLE 2—*Bovine Tubercle Bacilli (Ravenel Strain)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
102	½ hour	0	0	0	20	5	15
103	½ hour	0	0	0	10	12	12
96	2 weeks	0	0	1	100	60	500
97	2 weeks	0	0	0	0	300	30
98	4 weeks	0	0	0	0	30	200
99	4 weeks	0	0	0	250	20	150
109	8 weeks	2	0	0	1,000	70	150
110	10 weeks	50	0	0	1,000	60	1,250
111	10 weeks	25	0	0	5,000	80	350

TABLE 3—*Human Tubercle Bacilli (Jamaica 22)*

Rat	Time After Inoculation	Bacilli per Hundred Fields			Colonies per Milligrams of Tissue		
		Lung	Liver	Spleen	Lung	Liver	Spleen
74	½ hour	0	0	0	50	30	30
75	½ hour	0	0	0	100	15	90
76	2 weeks	0	0	0	0	3	3
77	2 weeks	0	0	0	1	10	120
79	4 weeks	0	0	0	150	15	20
112	8 weeks	0	0	0	200	5	40
113	10 weeks	0	0	0	500	3	7
114	10 weeks	0	0	0		0	7

used, as before, and, in addition, 9 rats were given the same quantity of Jamaica 22, a virulent human strain. The results are shown in tables 2 and 3.

Discrepancies are bound to occur when only small parts of organs are examined. In these experiments the specimens were taken from the same part of the organ in every rat, regardless of whether that part appeared to be diseased or not. The results doubtless would have been different if the specimens had been taken from those parts which on gross dissection appeared to have lesions. Errors in the counting of tubercle bacilli are exemplified by the findings in rats 100 and 101 of experiment 1. These animals were both killed half an hour after inoculation. By direct counting the tubercle bacilli in sections of the lungs, rat 101 was found to have more than twice as many bacilli as rat 100, yet the number of colonies grown from rat 100 was twice that from rat 101.

In counting 100 microscopic fields a very small volume of lung was studied, estimated as only 0.0106 cu. mm. Further, in specimens obtained a short time after inoculation the bacilli were for the most part clumped together in the small

blood vessels of the lungs. Later they were seen to have migrated into the pulmonary substance, and by about the third week most of them were lying within epithelioid cells. In the rats killed at six months they were in large numbers in epithelioid and giant cells scattered throughout the lungs. Microscopic examination did not reveal whether the organisms were alive or dead.

With the culture method a much larger volume of tissue was used, a suspension was prepared from 200 to 300 mg., and 1 mg. of this was planted on the culture medium. The liver and spleen could easily be ground in a mortar, but considerable difficulty was experienced with the lungs.

To assist the breaking up of the clumps, the sodium dihydrogen phosphate solution was used, but microscopic films showed that this procedure even after prolonged grinding was by no means always successful. Failure to break up clumps diminished the number of counted colonies. With this technic, the colonies grew well, and not more than a dozen of more than 400 culture tubes became contaminated.

In the experiments shown in tables 2 and 3 the counting method was valueless on account of the scarcity of the bacilli. On the other hand, in table 1, in those cases in which the bacilli were at all plentiful the figure representing the number of organisms per cubic millimeter (obtained by multiplying all the results by 100) was considerably larger than the number of colonies which grew from 1 mg. of tissue. This difference is perhaps explained by the failure to break up clumps in the process of grinding.

Tables 2 and 3 show that tubercle bacilli in small numbers are not demonstrable in the tissues by microscopic examination of sections, whereas cultures prepared from weighed quantities of the tissue show that they are present in considerable abundance.

The results when the cultural method was used clearly indicate that the Ravenel strain had multiplied very considerably in the lungs by the end of the eighth week. In general there was scant multiplication in the liver and the spleen. In some animals there was an increase in the spleen, but the figures for the liver showed little change. With the Jamaica 22 strain there was a tendency to multiply in the lungs. Unfortunately, no reading was possible on rat 114, owing to contamination, the liver and spleen of this animal suggested a slight diminution in numbers.

In experiments in which large (1 mg. in table 1) and small (0.01 mg. in table 2) quantities of bovine tubercle bacilli were injected intravenously into rats, cultures demonstrated that the tubercle bacilli steadily multiplied in the lungs. In the liver and spleen there was much less multiplication, these organs evidently inhibited but did not destroy the bovine tubercle bacilli. A somewhat similar relation was found by Lurie<sup>2</sup> in rabbits inoculated with bovine tubercle bacilli.

Notwithstanding the apparent resistance of the rat to tuberculous infection, tubercle bacilli may multiply with little apparent restraint in the lungs and in some instances in the liver or spleen. In association with scant antibody formation<sup>1</sup> there is multiplication of acid-fast bacilli within epithelioid cells with the production of lesions that recall those of leprosy.

#### SUMMARY

Tubercle bacilli, both bovine and human, introduced into the blood stream of the rat remain viable in the lungs, liver and spleen.

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<sup>2</sup> Lurie, M. B. *J. Exper. Med.* **55** 31, 1932.



Direct microscopic examination of sections is an unsatisfactory method for determining the number of living tubercle bacilli and fails to demonstrate them when few are present. Counting of colonies in cultures made with weighed quantities of tissue, though subject to error caused by clumping of bacilli, determines more exactly the relative number of micro-organisms in internal organs at intervals after infection.

Bovine tubercle bacilli on arrival in the lungs of rats after intravenous inoculation increase almost continuously so that great numbers are present after from two to six months. Human tubercle bacilli on arrival in the lungs after intravenous injection multiply, but the increase which occurs is much less than that with the bovine type.

Bovine or human tubercle bacilli that reach the liver and spleen after intravenous inoculation persist and in a few instances multiply, multiplication is usually inhibited, however, so that after six months the average number is less than that at an earlier period.

# Case Reports

## METASTATIC ADENOCARCINOMA IN THE LOWER JAWBONE

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As far as we have been able to ascertain, the histologic aspects of a metastatic tumor in the jawbone have been reported only once. The reason for this is not that such metastases are rare but rather that the jawbones are almost never included in an autopsy or in a microscopic study of postmortem specimens. This scarcity of published material was one of the considerations that prompted the present report. A second was the possible correlation between the recent experimental work with transplantation of the epithelium of the urinary tract and the osteogenic properties of carcinoma of the prostate.

Hofer<sup>1</sup> and Euler<sup>2</sup> reported the changes in bone in the presence of primary malignant tumors of the jaws. Hofer was particularly concerned with the question as to which cells are responsible for the resorption of bone. However, in none of the cases reported by these two authors were there metastases from distant tumors. Skillen,<sup>3</sup> on the other hand, described the changes in the maxilla and mandible of a woman of 40 years with carcinoma of the breast. Most of the jawbone had been invaded by tumor metastases, in places the alveolar bone had been destroyed, and tumor cells lay in contact with the tooth surface. Many good photomicrographs illustrated this. Throughout the jaws, simultaneous resorption of bone and formation of new bone were found. No detailed clinical history of the patient was given, however, and no conclusions were drawn as to the possible significance of the changes observed in the bone.

### REPORT OF A CASE

A white man 63 years old was well until about six months before death. At that time he experienced increasing pain in the joints, which made movement difficult and impaired his gait. Pain developed in the chest. He became progressively weaker and lost 45 pounds (20 Kg) in the last three months. On his admission to the hospital a diagnosis of inoperable carcinoma of the prostate with extensive metastases was made. The patient died of bronchopneumonia.

*Autopsy*—The postmortem examination disclosed adenocarcinoma of the prostate gland with metastases to the urinary bladder, rectal mucosa, liver, lungs, spleen, right and left inguinal lymph nodes, periaortic and iliac lymph nodes, a

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1 Hofer, O. Ztschr f Stomatol **23** 522, 1925

2 Euler, H. Deutsche Monatschr f Zahnhe **43** 701, 1925

3 Skillen, W. G. J Am Dent A **17** 1678, 1930

femur, a lumbar vertebra, the sacrum and ribs. There was confluent bronchopneumonia of the left lung. Benign cortical adenoma was present in both adrenals. There were moderate hypertrophy of the heart and syphilitic aortitis. Emaciation was marked.

*Gross Specimen*—The specimen consisted of the upper and lower jaws, which had been removed during the autopsy. The teeth were in poor condition, most of the anterior teeth were missing, and, of the posterior teeth, several had been destroyed by caries, so that only the roots were present. The roentgenograms taken before decalcification revealed no significant conditions of bone that could be interpreted as indicating changes due to a tumor metastasis.

*Method of Investigation*—The jaws were fixed in solution of formaldehyde U. S. P. and alcohol and decalcified in 5 per cent nitric acid. Then the upper



Fig 1—Bone and bone marrow in the upper jawbone of a man who died of carcinosis originating from an adenocarcinoma of the prostate. A, gelatinous and fibrous bone marrow with a few fat cells, B, red bone marrow, C, compressed original connective tissue of the fibrous marrow, D, osteoclasts and Howship's lacunae. Magnification, 65.

jawbone was divided into eleven parts and the lower jawbone into nine parts, and the parts were embedded in celloidin (a preparation of pyroxylin). The sections were stained with hematoxylin and eosin.

*Histologic Observations*—(a) Upper Jawbone. The upper jawbone was entirely free of tumor metastasis. The bone marrow consisted of all three types of marrow. Most of the marrow spaces contained adult fat marrow. In addition, there was a considerable amount of fibrous marrow, and scattered through the upper alveolar process were small islands of red cellular marrow. Most of the bone surface was aplastic, only in some areas, where the red marrow seemed to have been on

the increase, was there lacunar resorption of the surrounding bone trabeculae. A typical area of this kind, taken from the upper jawbone, is shown in figure 1. At *A* there is fibrous marrow with occasional fat cells embedded in an edematous, gelatinous-appearing matrix. At *B* is an island of red marrow, probably of erythroblastic nature, it is surrounded by a narrow margin of fibrous tissue, *C*, which appears to be the displaced residue of the original fibrous marrow. On the surface of the surrounding bone, numerous osteoclasts, *D*, are located in Howship's lacunae.

These histologic changes in the upper jawbone may be interpreted as secondary reactions to the emaciating disease of this patient. Fibrous changes in the marrow of the jawbones are known to accompany chronic dental infection of the type present in this jawbone. Gelatinous degeneration of the bone marrow is brought about by the cachexia accompanying malignant disease. Islands of red marrow are almost always found in adult jawbones. However, in this person the progressive character of the change in the red marrow suggests a correlation to the secondary anemia associated with carcinomatosis.

(*b*) Lower Jawbone. The lower jawbone was of particular interest because of the extensive invasion of the metastasizing carcinoma. It was impossible to determine from which side or along what pathway the invasion took place, because the bone was rather uniformly infiltrated throughout the mandible. A general view of an anteroposterior section through the lower left third molar and the root fragments of the second molar is shown in figure 2, upper part. Most of the bone marrow, except for the area at the alveolar crest, is densely infiltrated with tumor masses. The tumor has also invaded the mandibular canal, *MC*, and in some places has come in contact with the tooth surface. Along the mandibular canal, the tumor has grown from the adjacent cancellous bone through multiple resorption channels toward the inner bone surface of the canal (fig 2, lower part). Here its growth appears to have been temporarily checked by the dense fibrous sheath, *C*, surrounding the mandibular vessels and nerves, so that it spreads along the bone surface and has not yet invaded the nerve-vessel bundle proper. In the vicinity of the tumor there is formation of new bone, *NB*. The border between original and new bone is indicated by a dark cementing line. The nature and significance of the new bone will be discussed in connection with some of the later illustrations.

Figure 3, upper part, shows a field in the compact bone of the outer cortical plate of the mandible in the region of the right first molar. The larger haversian canals contain tumor masses, which follow the general course of the tissue spaces. Along the walls of the invaded haversian canals, new bone is being laid down. This is particularly plain in the large haversian canal, *H*., in which a mass of tumor cells is almost completely encircled by new, partly still uncalcified bone, *B*. On higher magnification of one of the haversian canals, the character and arrangement of the tumor cells are visible (fig 3, lower part). The tumor consists of columnar cells, which in some areas are arranged definitely in the shape of acini. The surrounding bone is covered with flat osteoblasts and with a layer of newly formed, uncalcified bone matrix (osteoid, *D*).

The osteoplastic character of this carcinoma is particularly evident in areas like the one illustrated in figure 4, upper part, which shows the inside of a larger marrow cavity that is being invaded by the tumor. The original bone surface can be clearly recognized by a cementing line (*A*). Alongside the entire bony wall of the marrow space, abundant formation of new bone has taken place. This bone has been laid down in delicate trabeculae, the surface of which is densely

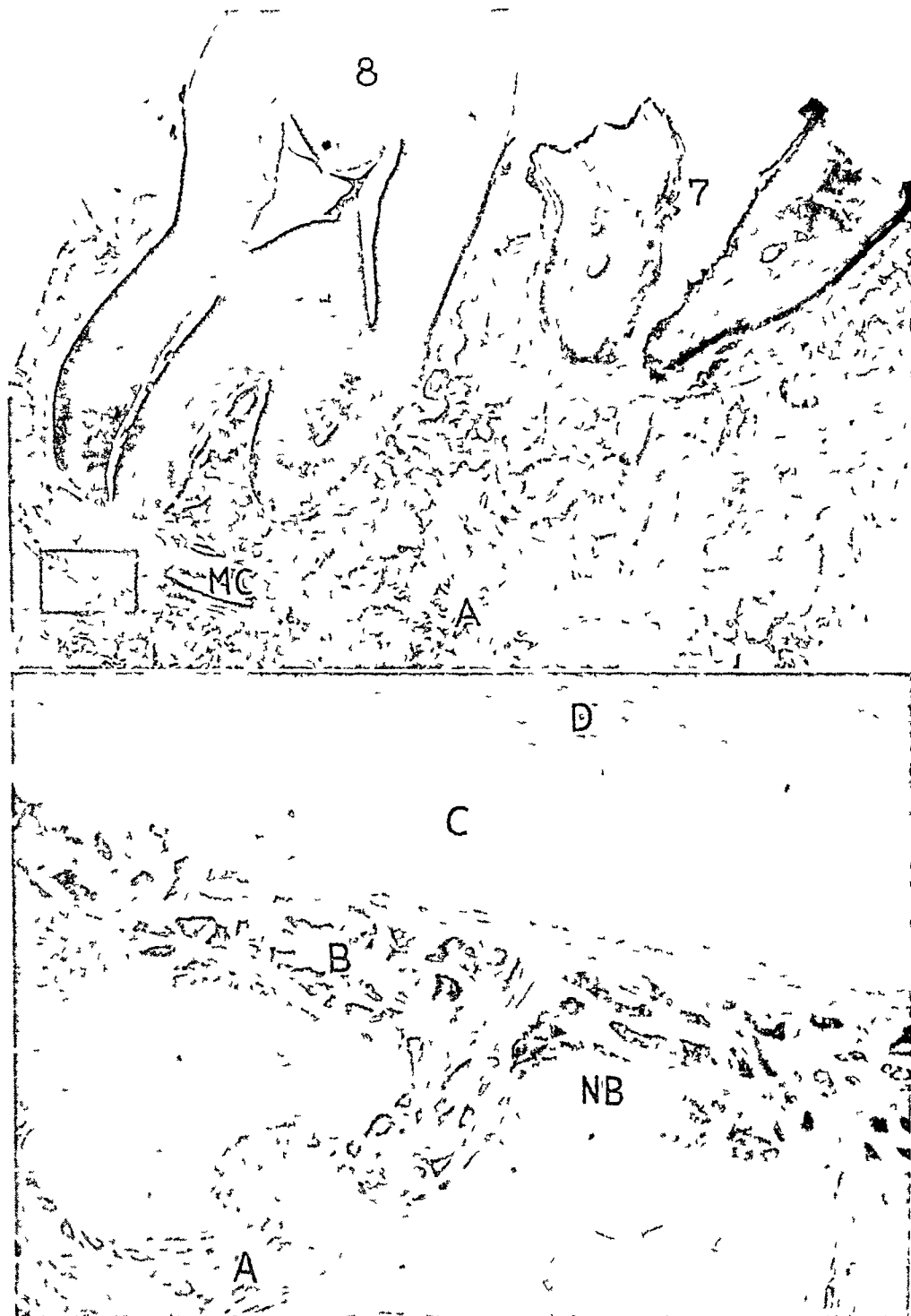


Fig 2—Upper Anteroposterior section through the lower left third molar (8) and the root fragment of the second molar (7) Note the diffuse carcinomatous infiltration of the bone marrow (A) and the invasion of the mandibular canal (MC) by tumor cells The area marked with a square is shown below under higher magnification Magnification, 4

Lower Carcinoma breaking through the bone wall of the mandibular canal A, carcinoma in the fibrous bone marrow, B, carcinoma spreading beneath the endosteum lining the mandibular canal, NB, new bone in the vicinity of the tumor, C, fibrous sheath of mandibular vessels and nerves, D, mandibular nerve Magnification, 36

beset with osteoblasts. There seems to be a definite tendency on the part of the bone to surround the islands and pegs of tumor cells. This is shown in a high magnification of the newly deposited bone (fig 4, lower part, which is the area

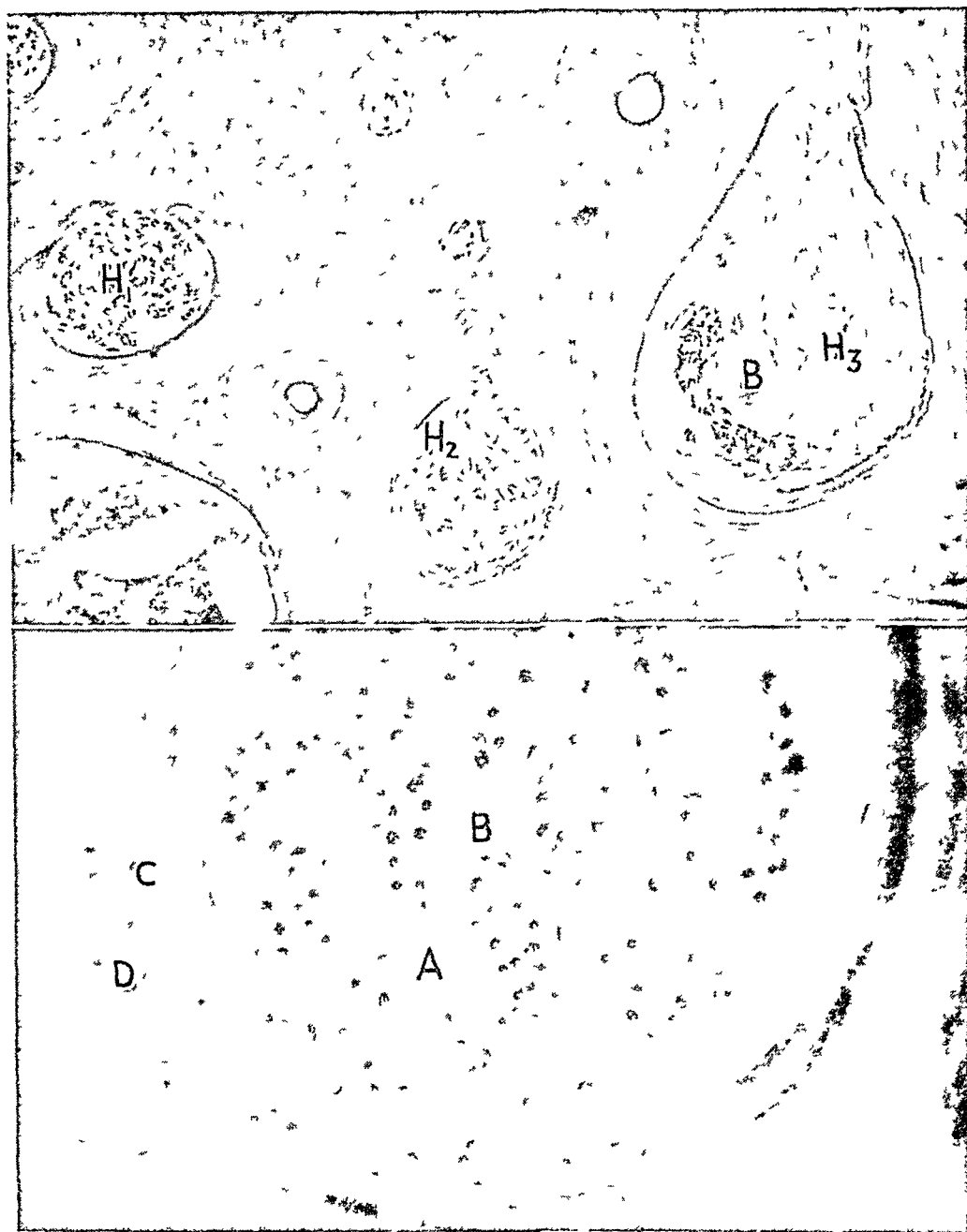


Fig 3—Upper Carcinoma invading the compact bone on the labial plate of the mandible in the first molar region.  $H_1$ ,  $H_2$ , Haversian canals containing tumor acini,  $H_3$ , a large bone canal containing fibrous marrow and being invaded by tumor cells,  $B$ , new bone surrounding the tumor cells. Magnification, 65.

Lower High magnification of Haversian canal,  $H_2$ , shown in upper part.  $A$ , acini of the adenocarcinoma,  $B$ , connective tissue stroma of the tumor,  $C$ , osteoblasts,  $D$ , uncalcified new bone matrix. Magnification, 275.

marked by a square in fig 4, upper part) At *C*, cross sections of small clusters of carcinoma cells are visible They are surrounded by bone, most of which is still uncalcified Only in the center of the trabeculae has some calcification taken



Fig 4—Upper Portion of marrow cavity containing fibrous marrow *A*, connecting line indicating the original bone surface, *B*, tumor tissue, *C*, new bone trabeculae proliferating from the original bone surface toward the tumor Magnification, 32 The area marked with a square is shown below under high magnification

Lower High magnification of the area marked with a square above *A*, old bone, *B*, cementing line, *C*, tumor cells, *D*, new calcified bone, *E*, newly formed, still uncalcified bone, *F*, osteoblasts Magnification, 283

place, as indicated by the dark blue hematoxylin stain. The bone surface stains only pale pink with eosin. The widest layer of uncalcified bone matrix is at the ends of the trabeculae where the bone has been actively growing toward the tumor cells that have invaded the fibrous bone marrow.

## COMMENT

It is generally known that in about 70 per cent of all cases of carcinoma of the prostate the lesion is accompanied by skeletal metastases (Ewing<sup>4</sup>, Geschickter and Copeland<sup>5</sup>, Warren, Harris and Graves<sup>6</sup>). All of these are of osteoplastic character. Although areas of resorption can usually be observed microscopically, the formation of bone far exceeds the destruction of bone. Other types of carcinoma also occasionally produce bone-forming metastases, but carcinoma of the prostate ranks first among those with osteoplastic metastases.

Several explanations have been offered for the osteoplastic reaction of the connective tissue to certain metastatic types of carcinoma. Geschickter and Copeland suggested that the invasive power of the tumor is low enough to allow the proliferating bone to keep pace with the invasion. Thus, they look on this process as a form of protective reaction on the part of the bone. Recklinghausen (cited by Ewing) attributed the formation of bone to a low grade chronic inflammatory reaction of the connective tissue. He spoke of carcinomatous osteitis, caused by obstruction and stasis in the capillaries of the bone marrow, produced by tumor emboli, which resulted in reactive growth of the connective tissue and bone. Axhausen<sup>7</sup> emphasized the possibility, suggested first by Recklinghausen, that the carcinoma cells liberate substances that act as a chemical irritant and thus cause proliferation of bone. This opinion is also given by Goetsch,<sup>8</sup> who uses it as an explanation for the occurrence of bone formation at a considerable distance from the actual tumor tissue.

Recent experimental work of Huggins<sup>9</sup> showed that transplantation of epithelium from the urinary tract into certain susceptible connective tissue areas results in the extraskeletal formation of bone. The transplanted epithelium itself does not become ossified, but bone develops in the connective tissue adjacent to the actively growing epithelial tissue. In view of these observations, it might be suggested that the epithelial cells of the carcinoma of the prostate, being epithelium of the urinary tract, retain the power to stimulate osteogenesis in susceptible connective tissue. There can be no doubt but that the connective tissue of the endosteum and bone marrow is more capable of reacting by the formation of bone than any other connective tissue of the body.

4 Ewing, J. *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1934.

5 Geschickter, C. F., and Copeland, M. M. *Tumors of Bone*, New York, American Journal of Cancer, 1931.

6 Warren, S., Harris, P. N., and Graves, R. C. *Arch. Path.* **22** 139, 1936.

7 Axhausen, G. *Virchows Arch. f. path. Anat.* **195** 358, 1909.

8 Goetsch, W. *Beitr. z. path. Anat. u. z. allg. Path.* **39** 218, 1906.

9 Huggins, C. B. *Arch. Surg.* **22** 377, 1931.



Recently Ashburn<sup>10</sup> reported the occurrence of bone and cartilage within a primary carcinoma of the prostate. He referred to the work of Huggins as offering a possible explanation of this observation.

#### SUMMARY

This report deals with the extensive metastasis of an adenocarcinoma of the prostate to the mandible in a man of 63 years. The connective tissue and bone marrow reacted to the invading tumor by extensive formation of new bone. This bone was laid down in the immediate vicinity of the neoplasm, and part of it is still uncalcified.

There is a possible correlation between the osteoplastic reaction to this carcinoma and the experimental observations of Huggins concerning the osteoplastic property of epithelium transplanted from the urinary tract. It is suggested that the cells of this carcinoma retained their power to stimulate formation of bone in susceptible connective tissue.

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10 Ashburn, L. L. Arch Path 28 145, 1939

## PARAFFINOMA OF THE LUNG WITH SECONDARY TUBERCLE-LIKE LESIONS IN THE LIVER AND SPLEEN

HENRY PINKERTON, M D, AND VICENTE MORAGUES, M D, ST LOUIS

Two fairly distinct types of lesion may be found as a result of the entrance of liquid petrolatum into the lungs by way of the trachea

In 1925 Laughlin<sup>1</sup> called attention to an acute pneumonic process with peculiar histologic features, resulting from the aspiration of liquid petrolatum. This lesion was accompanied by bacterial infection but not by conspicuous fibrosis, the consolidation of the lung tissue being caused largely by the accumulation of oil-laden phagocytes and other inflammatory cells in the alveoli and alveolar walls

In 1927 Pinkerton<sup>2</sup> reported multiple firm fibrous nodules in the lungs of a 6 year old boy and showed that these tumor-like nodules were composed of large globules of liquid petrolatum, embedded in dense hyaline fibrous tissue. It was pointed out that these nodules were comparable to the so-called paraffinomas produced by subcutaneous injections of liquid petrolatum. The identification of the oil was based on the facts that it did not blacken with osmic acid and that it was insoluble in absolute alcohol

Although conditions in many respects intermediate between these two types have been described (Pinkerton<sup>2</sup>), it has recently been pointed out by Ikeda<sup>3</sup> that the recorded lesions resulting from the aspiration of oily or fatty substances fall more or less sharply into the two groups described. The latter worker suggested that the early pneumonic process be called "lipoid pneumonia of the infantile type" and the late cicatricial nodular lesion "lipoid pneumonia of the adult type," or paraffinoma of the lung (if liquid petrolatum is the material involved). This classification, though not entirely satisfactory, seems to be of considerable practical value

Approximately 85 cases of the infantile type of lesion as defined in the foregoing paragraph had been reported up to 1936 (Ikeda<sup>3</sup>). The majority of the patients were infants and children. Pinkerton<sup>2</sup> showed that oily and fatty substances other than liquid petrolatum, notably cod liver oil, olive oil and milk fat, may be responsible for lesions of this general nature, and this has been substantiated by later observations. The latter worker<sup>4</sup> also showed experimentally that the reaction to cod liver oil is characterized by specific histologic features, the most characteristic of which are the shredding of the oil and the intense acid-

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1 Laughlin, A J. *Am J Path* **1** 407, 1925

2 Pinkerton, H. *Am J Dis Child* **33** 259, 1927

3 Ikeda, K. *Arch Path* **23** 470, 1937

4 Pinkerton, H. *Arch Path* **5** 380, 1928

fast staining of the oil by the Ziehl-Neelsen method, a property which it acquires by oxidation in the alveoli. Other somewhat less reliable criteria for identifying other types of oil or fat were described.

The adult type of lesion as defined in a foregoing paragraph (paraffinoma) has proved to be of less frequent occurrence, so far as one may judge by the cases reported, although it should be pointed out that both types are probably often overlooked by pathologists not specifically searching for them. A second case of this type was reported by Fischer-Wassels<sup>5</sup> in 1933, six years after the first instance. Ikeda<sup>3</sup> found that 14 additional cases were reported between 1933 and 1936, and added 5 cases of his own. In only 14 of these 21 cases, however, was the diagnosis confirmed by necropsy, the diagnosis in the other 7 depending on clinical and roentgenologic evidence alone.

In the original case of paraffinoma (Pinkerton<sup>2</sup>) liquid petrolatum was found in relatively small amounts in the reticular tissue of the spleen, where it had obviously been carried by the blood stream. In the case to be described here the left lung contained a single large paraffinoma, although the histologic picture in many other areas was much like that of the infantile type of lipoid pneumonia. The case is of particular interest because of the fact that the liver and spleen were studded with tubercle-like lesions, ranging up to 3.5 mm in diameter, which were found microscopically to be encapsulated masses of granular and globular material, consisting largely of liquid petrolatum. The significance of this observation will be discussed later.

#### REPORT OF A CASE

The patient was a 61 year old white man. In this man, at the age of 41, following a febrile illness diagnosed as influenza, typical paralysis agitans developed. It persisted up to the time of death. During the fifteen year period preceding death, precordial and substernal pain were complained of periodically, with progressive dyspnea. Swelling of the ankles was noted during the last year of life. Eight years before death nephrolithiasis was diagnosed, and four years later a stone was removed from the right ureter. During the last five years of life there were frequent attacks of pharyngitis and bronchitis, with the collection of large amounts of mucus in the throat, and increased salivation, not relieved by atropine.

Dec 8, 1938, the patient had a chill, with pain in the left side of the chest. Auscultation disclosed rales in both lungs, and roentgen examination showed a picture which was interpreted as consolidation of a portion of the lower lobe of the left lung. Sputum culture showed *Pneumococcus* type VI, and two injections of 20,000 units of type VI serum were given. The red blood cell count was 3,140,000, the white cell count, 12,450, with 70 per cent neutrophils. The urine showed no albumin, sugar, casts or cells. Death occurred December 13.

Unfortunately, information relative to the use of liquid petrolatum was not obtained from the patient, since the nature of the pulmonary condition was not suspected during life. Subsequent questioning of friends and attending physicians brought out the fact that liquid petrolatum had been taken freely by mouth over a period of several years for the relief of severe constipation.

*Necropsy*—The body was emaciated, and there was pitting edema of the ankles and on the inner aspects of the thighs. There were fibrous adhesions between

5 Fischer-Wassels, B. Frankfurt Ztschr f Path 44 412, 1933

several loops of small intestine. The heart was normal in size. The descending branch of the left coronary artery was markedly calcified but not occluded, and the aorta showed advanced atheromatous changes throughout its entire length.

A layer of shaggy, yellowish white fibrinous exudate covered the lateral and diaphragmatic visceral pleura of the lower lobe of the left lung. In the central portion of this lobe a hard mass was palpated. On section, the mass was seen to be sharply circumscribed, irregularly nodular and roughly spherical in shape. It was 4 cm in the greatest diameter. Its central third was grayish black, from carbon pigment, and almost woody in consistency, while peripherally the tissue was grayish white and somewhat less firm. The cut surface of the mass bulged slightly above the surrounding lung tissue. Bands of scar tissue, radiating outward from the dense central core, divided the peripheral portion of the mass into several distinct secondary nodules, averaging about 1 cm in diameter. In appearance and consistency this nodular mass was suggestive of primary or metastatic carcinoma.

Medial to the large fibrous mass was an area of firm grayish consolidation, interpreted as confluent bronchopneumonia. This area represented about one fifth of the entire volume of the lower lobe of the left lung and extended downward to the diaphragmatic surface. Laterally and somewhat anteriorly there was an irregular abscess cavity, 3 cm in the greatest dimension, lined with fibrinous exudate and surrounded by a heavy fibrous wall 2 to 3 mm in thickness. This abscess was at a slightly lower level than the fibrous mass and separated from it by a wall of partially crepitant lung tissue about 1 cm in thickness. From the abscess a sinus tract led to the pleural surface, which, as mentioned earlier, was covered with fibrinous exudate. The relative positions of the paraffinoma, the area of pneumonia and the abscess are seen in figure 1. There were several small areas of consolidation in the upper portion of the lower lobe of the left lung and also in the lower lobe of the right lung.

The liver was moderately enlarged. The external and cut surfaces were studded with firm, grayish white nodules resembling miliary tubercles. These varied in diameter from 0.5 to 3.5 mm, averaging 1.5 mm. In many areas three or four of these lesions were seen per square centimeter of cut surface.

The spleen was about one and a half times the normal size. The pulp was soft and studded with tubercle-like lesions similar to those described in the liver.

The pelvis of the right kidney contained a large brown concretion, irregular in shape and with prolongations into the calices. Nodules like those in the liver and spleen were not observed in either kidney at the time of the necropsy. Unfortunately the kidneys were not saved for more careful examination later on.

*Microscopic Examination*—Sections of the hard nodule in the lower lobe of the left lung showed the typical picture of paraffinoma (fig 2 *A* and *B*). This will not be described in detail, as it was entirely similar to instances previously reported by Pinkerton,<sup>2</sup> Ikeda<sup>3</sup> and others. Sections from the center of the nodule (fig 2 *A*) showed oil globules embedded in a dense hyaline keloid-like collagenous material. Peripherally, the connective tissue was less dense and contained many inflammatory cells, with frequent giant cells surrounding oil droplets (fig 2 *B*). The area of pneumonia and the abscess showed the characteristic microscopic pictures of these conditions, but oil-laden phagocytes were numerous throughout. Sections of smaller nodules from the upper part of the lower lobe of the left lung showed thickened alveolar walls lined with cuboidal epithelium, and oil-laden phagocytes were present in the alveoli and in the alveolar walls. To this, a picture of acute suppurative pneumonia was added in many areas. Sections from

the nodules in the lower lobe of the right lung were not labeled specifically, and as the right lung was not saved, no statement can be made regarding the nature of these lesions

The oil droplets in the lung tissue did not stain at all with osmic acid, and it may be said here that oil collected from the surface of the solution of formaldehyde in which the lung tissue was kept was found to be insoluble in absolute alcohol and completely resistant to saponification

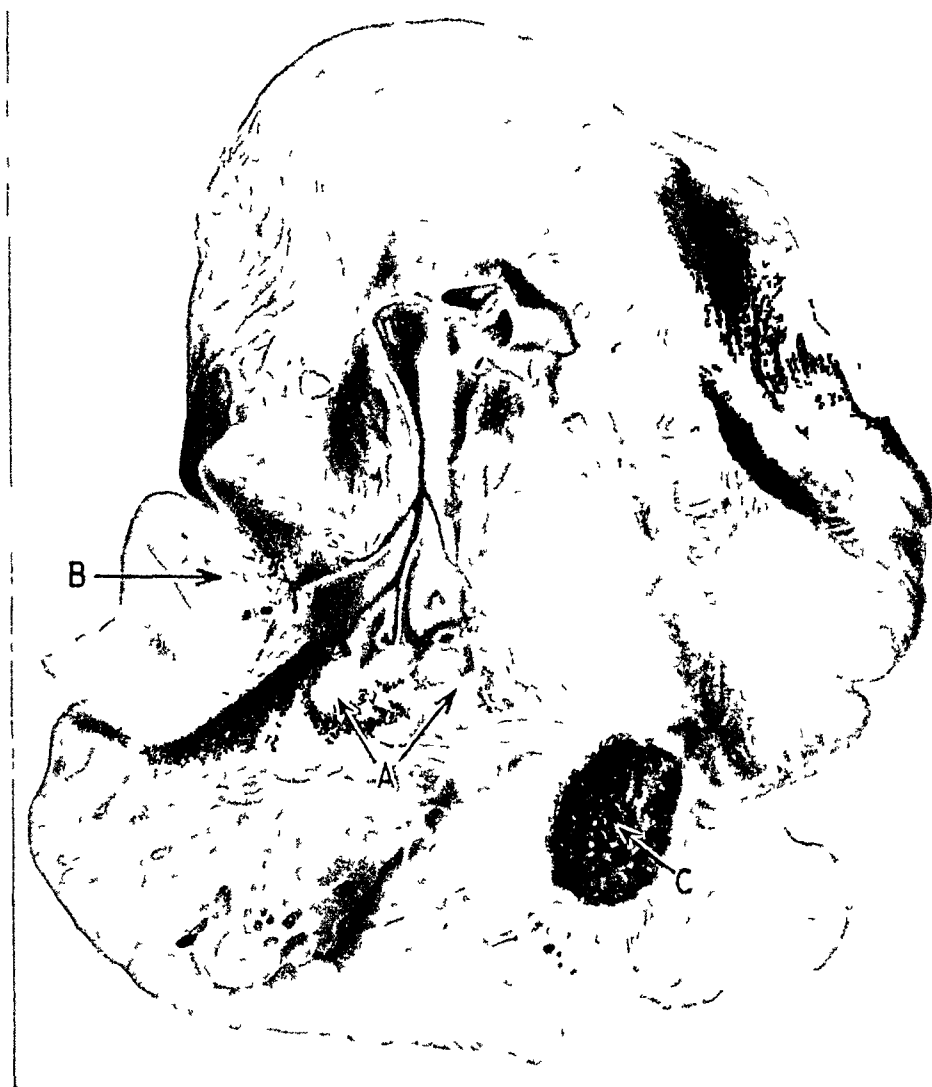


Fig 1—Drawing of the left lung, showing (A) the paraffinoma in the central portion of the lower lobe, (B) an area of pneumonic consolidation medially and at a slightly higher level and (C) an abscess cavity laterally and at a slightly lower level

Peculiar vascular lesions, illustrated in figure 3 A, were found in the arteries and veins in several sections of lung tissue. The lumens of these vessels contained globules of liquid petrolatum (as shown by the staining reactions) surrounded by fibrous tissue in such a way as to resemble normal adipose tissue. The picture was like that of organized mural thrombi, the material organized being oil rather

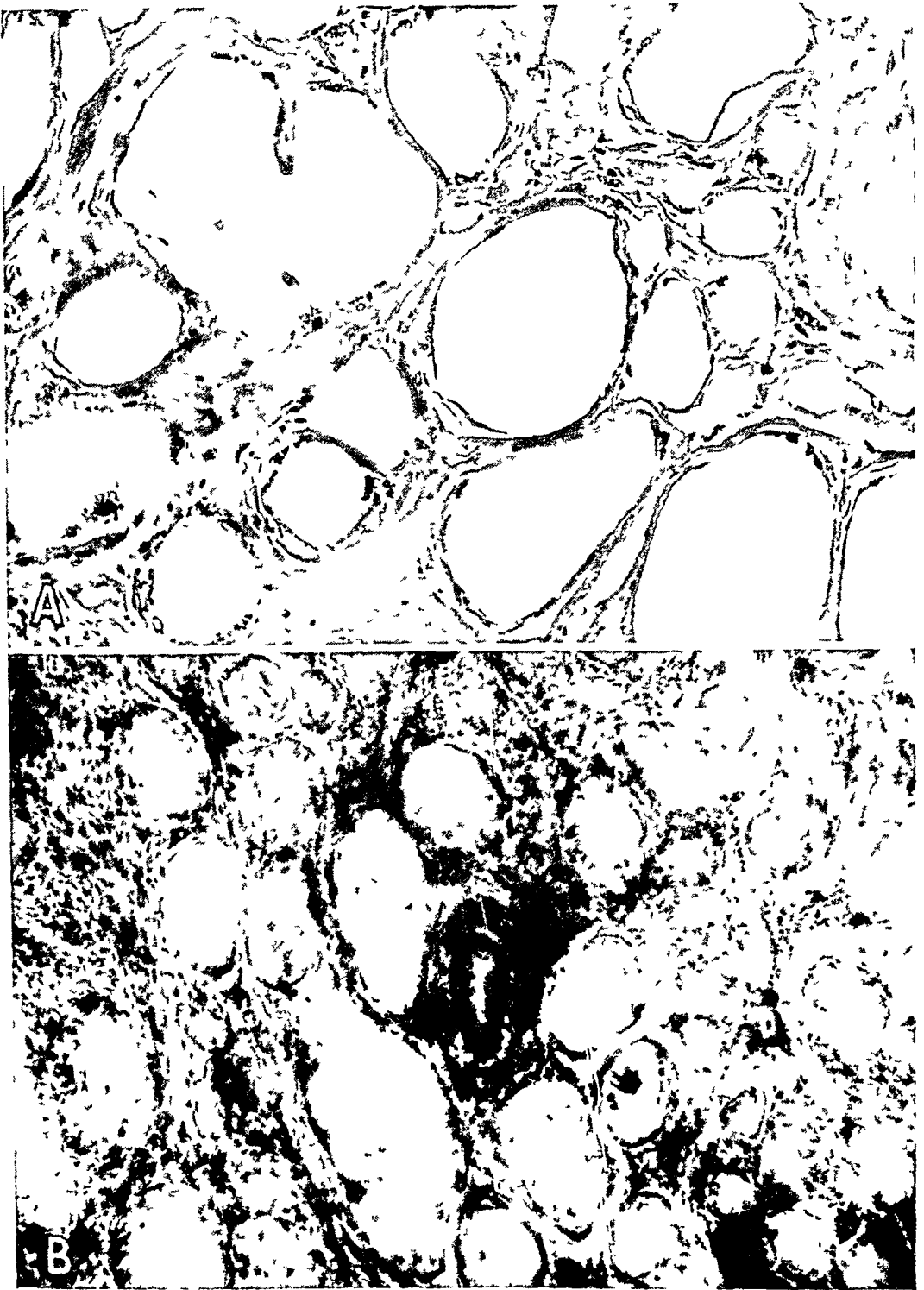


Fig 2—*A*, photomicrograph from a paraffin section of the lung, showing the histologic appearance of the dense central portion of the paraffinoma. Spherical masses of oil, represented here by empty spaces, are embedded in a dense hyaline connective tissue. Hematoxylin and eosin stain,  $\times 100$ . *B*, photomicrograph from a frozen section of the lung, representing the peripheral portion of the paraffinoma. The spherical masses of oil have been stained with scarlet red,  $\times 70$ .



Fig 3—*A*, thrombus-like lesion in a pulmonary arteriole, apparently resulting from organization of oil within the lumen of the vessel. Hematoxylin and eosin stain,  $\times 100$ . *B*, vacuoles representing droplets of liquid petrolatum in the reticular tissue of the spleen. Hematoxylin and eosin stain,  $\times 200$ .

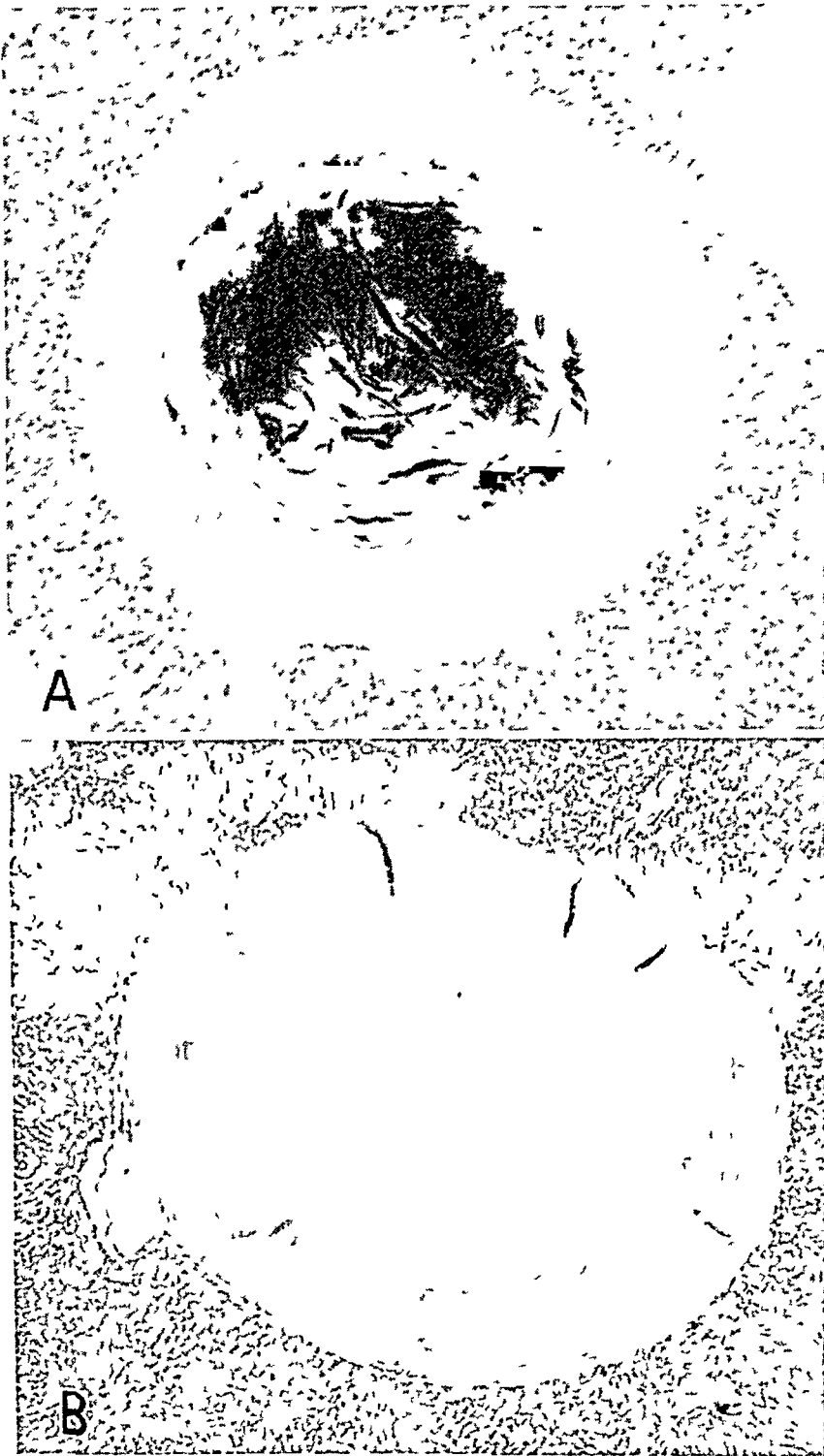


Fig 4—*A*, tubercle-like lesion of the liver, with calcium and cholesterol crystals in the central core. Hematoxylin and eosin stain,  $\times 100$ . *B*, large tubercle-like lesion with hyaline center in the liver. Hematoxylin and eosin stain,  $\times 50$ .



than fibrin. The mechanism of the production of these lesions is not clear, and it is difficult to understand how the oil gained access to the lumens of these vessels but it seems likely that these lesions may have been the starting point for oil emboli, which could have caused the peculiar nodular lesions in the liver and spleen. It is clear that these pulmonary lesions are not artefacts, since the organizing connective tissue was always continuous with that in the walls of the vessels.

The lesions in the liver and spleen were apparently identical, and scarlet red stains of frozen sections from the deeply pigmented bronchial lymph nodes revealed a number of similar lesions in these organs. The central portion of each of these nodules was composed of an amorphous mass of globular and granular material staining deep red with scarlet red but not blackening with osmic acid. Peripherally, each nodule was encapsulated by a wide zone of dense fibrous tissue. Many of the nodules contained precipitated calcium and cholesterol crystals, as well as finely emulsified oil (fig 4A). In addition to these encapsulated masses of oil, both the liver and the spleen contained large amounts of oil in the form of large globules and small droplets, sometimes intracellular and sometimes extracellular, often with surrounding giant cells (fig 3B). None of the oil droplets in the spleen stained with osmic acid. In the liver there was some infiltrated fat, which could easily be distinguished from the oil by the fact that it stained with osmic acid.

No evidence of oil deposition was found in four sections of kidney tissue. The kidneys showed focal areas of healed pyelonephritis but no evidence of glomerulonephritis or nephrosclerosis. Brain, pancreas, heart and adrenal tissue were also apparently free from oil deposits.

#### COMMENT

In this case the neurologic condition is undoubtedly of importance in explaining the aspiration of the oil. In several of the reported cases, however, the paraffinoma has occurred in a person without neurologic damage or other obvious factor predisposing to the entrance of oil into the trachea.

It seems clear beyond a reasonable doubt that the abscess, empyema and focus of bacterial pneumonia were all secondary to the paraffinoma, since all three occurred in close proximity to it. The constriction of bronchioles and blood vessels involved in the area of scar tissue may be regarded as a factor favoring bacterial infection. In this respect, the oil, with the associated fibrosis, may be considered as acting like any other foreign body.

The tubercle-like lesions in the liver and spleen, which were shown to be composed of encapsulated liquid petrolatum, are of particular interest. Such lesions have not previously been described. It seems probable that they originated in blood vessels, although this could not be definitely demonstrated. Their microscopic appearance was not suggestive of tuberculosis.

Young and co-workers<sup>6</sup> recently reported a pulmonary oil tumor in which severe renal damage is believed to have resulted from the metastatic deposition of oil in the glomeruli and blood vessels of the kidney. The aspirated material was believed to be cod liver oil, and the vascular origin of the lesions was obvious. The possibility that serious damage may be done to various organs by liquid petrolatum,

<sup>6</sup> Young, A. M., Applebaum, H., and Wasserman, P. B. J. A. M. A. 113: 2406, 1939.

cod liver oil or other types of oil gaining entrance to the circulating blood is worthy of further consideration, especially in view of the probability that such foreign substances may be absorbed from the intestine to some extent without chemical alteration

#### SUMMARY

A case of paraffinoma of the lung with terminal abscess formation, bronchopneumonia and empyema is described pathologically. The abscess was apparently secondary to constriction of a bronchiole involved in the cicatricial tissue of the paraffinoma. Organized masses of liquid petrolatum, resembling mural thrombi, were found within the pulmonary vessels. The liver and spleen were studded with tubercle-like lesions, 0.5 to 3.5 mm in diameter, which were found microscopically to be encapsulated masses of liquid petrolatum, with the addition, in some instances, of calcium and cholesterol crystals. These peculiar lesions in the liver and spleen were undoubtedly secondary to the paraffinoma and probably embolic.

## TOPHUS OF THE MITRAL VALVE IN GOUT

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While urate crystals may be deposited in numerous structures of the body in the course of chronic gout, they are rarely found in the endocardium. Contrary to the impression given by modern texts, authentic reports of endocardial or valvular tophi are few indeed. In some cases, traces of uric acid, enough to yield a positive reaction in the murexide test, simply impregnate an old calcareous deposit. For example, Coupland,<sup>1</sup> in 1873, reported before the London Pathological Society that at autopsy on a patient who had had gout for eighteen years he found that "the aortic valves were the seat of a deposit strikingly like the gouty deposits occurring in the neighborhood of joints and not at all resembling calcified vegetations." But on submitting the specimen for chemical analysis he received the report that "an indication of the presence of uric acid is obtainable by murexide test, but the bulk of the concretion is composed, as usual, of phosphate and carbonate of lime." Indeed, Lancereaux<sup>2</sup> reported a case of interstitial nephritis terminating in uremia, and though the patient showed no evidence of gout, uric acid was deposited on the mitral valve. Moore<sup>3</sup> published the complete autopsy protocols of 80 patients with chronic gout observed in St. Bartholomew Hospital, and in not a single instance was an endocardial tophus mentioned. Garrod<sup>4</sup> wrote, "I have carefully examined the deposits found on the valves of the heart and the atheroma from the aorta of several gouty patients who had extensive chalk-stones in different parts of the body, but have failed to discover the least trace of uric acid." He quoted Landerer, who found in a patient a white deposit on the inner surface of the aorta, said to consist chiefly of calcium phosphate and calcium carbonate with about 14 per cent uric acid, and Lobstein, who found in a patient a deposit on the mitral valve which had a similar composition. Duckworth,<sup>5</sup> in his treatise on gout, wrote that he knew of no authentic case of gouty endocarditis.

The case which we have observed is therefore of considerable interest.

### REPORT OF A CASE

A white man of 63 years, a painter, was admitted to the Third (New York University) Medical Division of Bellevue Hospital on February 13, in a stuporous

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From the Third (New York University) Medical Division and the Pathological Laboratories of Bellevue Hospital and the Department of Medicine of New York University College of Medicine.

1 Coupland, S. *Tr. Path. Soc. London* **24** 69, 1873.

2 Lancereaux, E. *Gaz. med. de Paris* **23** 187, 1868.

3 Moore, N. *St. Bartholomew's Hosp. Rep.* **23** 289, 1887.

4 Garrod, A. B. *Treatise on Gout and Rheumatic Gout (Rheumatoid Arthritis)*, ed. 3, London, Longmans, Green & Co., 1876, p. 204.

5 Duckworth, D. *A Treatise on Gout*, Philadelphia, Blakiston, Son & Co., 1889.

state, unable to give a detailed history. For the past twenty years he had noted large nodules over the elbows and knees with deformity and stiffness of the small joints of the hands, wrists and knees. During the past six years cheesy material escaped from ulcerated surfaces over both tibiae. The family, past medical and dietary histories were unobtainable.

On admission he appeared stocky and well nourished but apathetic and seriously ill. He was only slightly dyspneic, not orthopneic or cyanotic. Large tophi, several centimeters in diameter, were situated symmetrically just distal to the olecranon and patella. The olecranon and prepatellar bursae, as well as those over the dorsal aspects of both wrists, were distended, irregular and cystic, although not tender. The hands were clawlike and showed interosseous wasting, ulnar deviation of the fingers, fusiform swelling of metacarpophalangeal and of several proximal interphalangeal joints. The extensor tendon sheaths of several fingers were distended and fluctuant. Numerous tophi were distributed over both hands near the digital articulations. Paronychia suppuration of one finger and one toe was noted (fig 1A). There were no tophi in the auricular cartilages.

On the anterior surface of the middle third of each tibia appeared an irregular deep ulcerated area, about 5 cm in diameter, discharging blood and white cheesy material (fig 1B). The latter was soft, not gritty, and a murexide test was positive. Microscopically, it consisted of long needle-like urate crystals.

The eyegrounds showed sclerotic vessels with arteriovenous compression and irregular areas of retinal degeneration. The cardiac apical impulse was not palpable, the rhythm was regular and the rate 100 per minute. No gallop or murmurs were noted. There were no signs of heart failure. The blood pressure was 200 systolic and 98 diastolic. An electrocardiogram presented a low, diphasic T in lead I and sinus tachycardia, with a rate of 110 per minute. The rest of the physical examination gave negative results. The temperature was irregular, fluctuating between 99 and 103 F.

The specific gravity of the urine varied from 1.010 to 1.015, a trace of albumin was consistently present, and there were 10 to 15 red blood cells and 5 to 10 white blood cells per high power field, and also finely granular casts. The blood showed 3,150,000 red cells, 14,000 white cells, with a normal differential formula, and 70 per cent hemoglobin. The blood nonprotein nitrogen varied from 112 to 150 mg and uric acid from 7.3 to 9.0 mg per hundred cubic centimeters (Folin method), the blood creatinine level was 2.5 mg per hundred cubic centimeters. The roentgen findings were characteristic of gout.

The patient followed a progressively downward course, becoming irrational, delirious, incontinent and finally comatose. He died thirteen days after admission. The clinical diagnosis was gout and chronic diffuse glomerular nephritis.

*Autopsy*—Autopsy was performed within twenty-four hours after death. The external appearance conformed to the clinical description given.

The heart weighed 570 Gm and showed preponderant left ventricular hypertrophy. Close to the free border of the posterior mitral leaflet appeared a sharply circumscribed concretion, 4 cm long and about 0.5 cm thick. Material removed from this concretion consisted of long needle-like crystals resembling those of sodium urate. There were no endocardial ulcerations or vegetations. Within the anterior mitral leaflet a few yellowish atheromatous plaques were seen. The tricuspid, pulmonary and aortic valves were normal. The coronary arteries showed diffuse sclerosis with mild narrowing. The aorta showed moderate atherosclerotic changes.

The kidneys weighed 150 Gm each and were pale gray. The capsule was adherent and could not be stripped without adherent parenchyma. The surface was made up of fine and coarse nodules with an occasional large cyst.

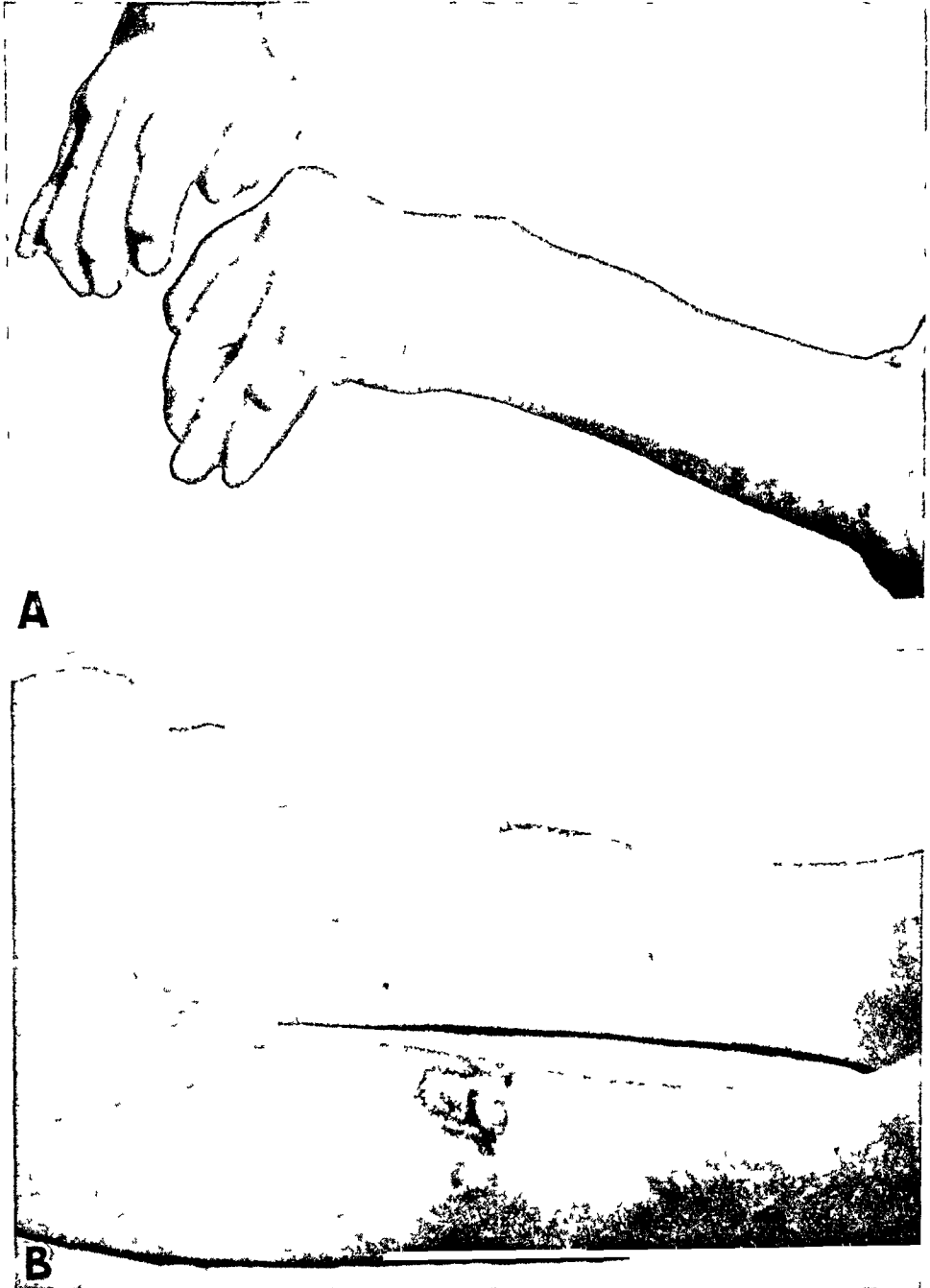


Fig 1—*A*, patient's upper extremities. Note the tophus at the left elbow. *B*, patient's lower extremities. Note prepatellar bursae and ulcers discharging uric acid crystals.

On cut sections no sharp demarcation between cortex and medulla was present. Numerous cysts 0.5 cm in diameter were noted. There were several pinhead and

slightly larger grayish white foci in the medullary rays. The remaining structures were normal on gross examination.

*Microscopic Observations*—Sections were taken through the posterior mitral leaflet. These were fixed in paraffin without decalcification and stained with hematoxylin and eosin. The posterior leaflet showed slight fibrous thickening of the proximal one-third and bulbous enlargement of the distal two-thirds. In the latter were large circumscribed masses of amorphous eosinophilic material, surrounded by fibrous tissue, in which were many multinucleated giant cells of the foreign body type, histiocytes, a few lymphocytes, plasma cells and fibroblasts. There were capillaries and thick-walled vessels in the distal part of the leaflet surrounding the concretions. There was no vascularization of the proximal portion of the leaf-

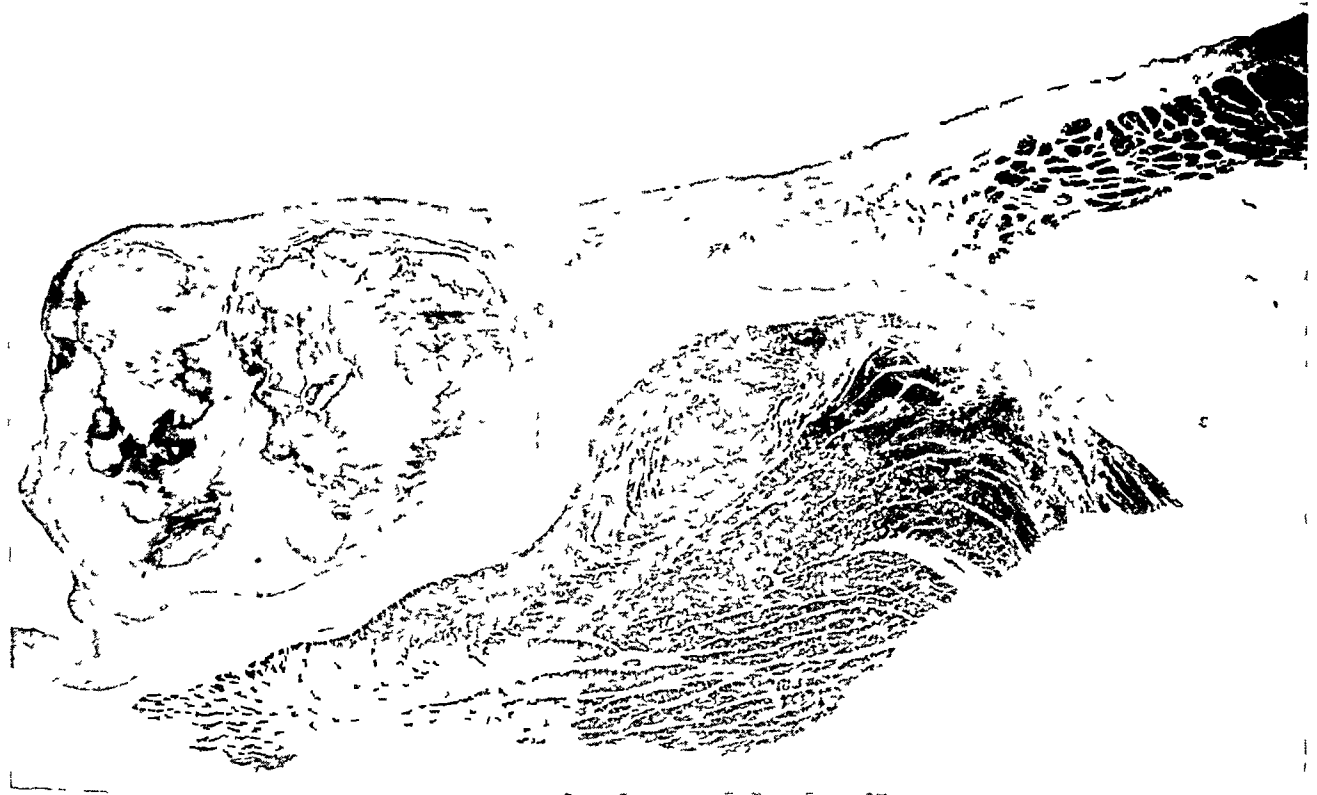


Fig 2—Gouty tophus embedded in posterior mitral leaflet,  $\times 12$

let. The overlying endocardium was intact. The annulus showed slight fibrous thickening with radiating interfascicular extensions. The endocardium of the posterior left auricular wall appeared normal.

Sections through the anterior mitral leaflet showed slight fibrous thickening of the senescent type, without vascularization or inflammation.

In the myocardium there was slight interfascicular fibrosis radiating from some of the medium-sized branches of the coronary arteries. The smaller coronary arteries showed definite intimal thickening.

The aorta showed marked intimal thickening and large atheromas. The arterioles of the spleen and pancreas presented marked intimal hyaline thickening.

Sections through the kidney showed diffuse alterations. The glomeruli were only slightly reduced in number, but few normal glomeruli were present. The majority showed slight thickening of the basement membrane with simplification

of the tuft. A smaller number showed varying degrees of hyalinization of tuft and Bowman's capsule. The interstitial tissue showed a slight diffuse increase with focal lymphocytic infiltration. The afferent glomerular arterioles presented pronounced subendothelial hyaline thickening, and the interlobular and arcuate arteries exhibited marked intimal hyperplasia with diminution of the lumens of the vessels. There were occasional small adenomas in the cortex, and in the medulla were several miliary and larger foci of amorphous eosinophilic material, surrounded by foreign body giant cells.

*Pathologic Diagnosis*—The final diagnosis was numerous subcutaneous tophi, a urate concretion in the posterior mitral leaflet, coronary arteriosclerosis, hypertrophy and dilatation of the cardiac chambers, atherosclerosis and diffuse arteriosclerosis of the aorta and visceral vessels, advanced arterial and arteriolar nephrosclerosis with urate deposits in the renal medulla and cortical adenomas, lobular pneumonia, pulmonary edema and pulmonary arterial thrombosis or embolism.

*Chemical Analysis*—(Dr Isidor Greenwald and Dr Solomon H. Rubin) The material removed from the mitral tophus was submitted to the Benedict and Franke test for uric acid, as well as to the murexide test, both of which were strongly positive. The sample also contained cholesterol. No calcium or phosphates were present. The material was insufficient for quantitative studies.

#### COMMENT

There seems to be enough evidence to warrant the conclusion that the concretion encountered in the posterior mitral leaflet was a tophus of uric acid crystals. This conclusion is based on the microscopic appearance of the crystals, the absence of any basophil material in undecalcified sections of the mitral valve and the chemical analysis, which showed presence of uric acid and absence of calcium. It is noteworthy that the concretion was situated in a valve showing little, if any, evidence of previous inflammatory changes.

Pathologic alterations of the type noted in the kidney are not unusual in cases of chronic gout. They have been described by many observers. Schnitker and Richter<sup>6</sup> reviewed 55 cases of gout studied at the Peter Bent Brigham Hospital, Boston, and found that 17 of the patients showed clinical evidence of nephritis, 5 died in uremia.

While the diagnosis of gout in this case offered no difficulty, it is interesting to note that many of the clinical signs resembled those frequently seen in rheumatoid arthritis.<sup>7</sup>

#### SUMMARY

A case of chronic gout with a uric acid tophus deposited in the mitral valve is reported. No clinical signs of this unusual lesion were present.

6 Schnitker, M. A., and Richter, A. B. *Am J M Sc* **192** 241, 1936.

7 Ludwig, A. O., Bennett, G. A., and Bauer, W. *Ann Int Med* **11** 1248, 1938.

# Laboratory Methods and Technical Notes

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## BIEBRICH SCARLET-PICRO-ANILINE BLUE A NEW DIFFERENTIAL CONNECTIVE TISSUE AND MUSCLE STAIN

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In the course of current studies on the staining of connective tissue, a very successful combination, based on the technics of Van Gieson, Malloy and Masson, was encountered. This technic, which may be carried through in less than twenty minutes on paraffin sections of routine material fixed in solution of formaldehyde, demonstrates basement membranes in renal glomeruli and intestinal mucosa and also much of the reticulum of lymphadenoid structures, and stains brilliantly the usual connective tissues in sharp contrast to muscle and cytoplasm.

### DESCRIPTION OF TECHNIC

Paraffin sections of material fixed in solution of formaldehyde, U S P (1 part to 4 parts or 1 part to 9 parts water), Orth's fluid or Zenker's fluid are deparaffinized and brought to 80 per cent alcohol as usual. The material fixed in Zenker's fluid is treated with iodine and then with thiosulfate as usual.

1 Stain five minutes in Weigert's acid-iron chloride-hematoxylin or other similar iron-hematoxylin stain.

2 Wash in water.

3 Stain four minutes in a solution made as follows: Biebrich scarlet, 0.2 Gm., glacial acetic acid, 1 cc., and distilled water, 100 cc.

4 Rinse in water.

5 Stain four to five minutes in a fluid made as follows: aniline blue W S, 0.1 cc., and saturated aqueous picric acid, 100 cc.

6 Wash three minutes in 1 per cent acetic acid in water.

Dehydrate, clear and mount. In this laboratory an acetone, acetone-xylene, xylene, salicylic acid balsam series is used.

Red corpuscles are orange scarlet, muscle deep salmon pink, cytoplasm gray to pink, nuclei black, basement membranes, reticulum and finely fibrillar connective tissue deep blue, and coarse and hyalinized connective tissue basically blue but perhaps showing red-violet areas.

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From the Division of Pathology, National Institute of Health



## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—Lauren V Ackerman, of the University of California Medical School, has been appointed pathologist to the Ellis Fischel State Cancer Hospital, Columbia, Mo

The Committee on Scientific Research of the American Medical Association has awarded a grant to L J Meduna, F J Gerty and V G Urse, Loyola University, Chicago, for a study of the biochemical changes in patients with schizophrenia under treatment with metrazol, which was originated by Dr Meduna

William H Taliaferro, of the University of Chicago, Karl F Meyer, of the University of California, James B Murphy, of the Rockefeller Institute for Medical Research, Stephen W Ranson, of Northwestern University, R E Shope, of the Rockefeller Institute, Princeton, George W Corner, of the University of Rochester, and Carl F Cori, of Washington University, St Louis, have been elected to membership in the National Academy of Sciences

Calvin B Coulter, associate professor of pathology in the Long Island College of Medicine, Brooklyn, and bacteriologist in chief at Kings County Hospital, has died at the age of 52 years

**Award**—W H Sebrell, surgeon, United States Public Health Service, shares in the Mead Johnson & Company award for "the most outstanding work on vitamin B complex in North America in 1939"

**Fellowships**—The Finney-Howell Research Foundation, Baltimore, has renewed seven fellowships for research on the nature and treatment of cancer and has awarded seven new fellowships for the same purpose. The closing date for filing applications for awards for 1941 is January 1 next

**Society News**—The sixth International Congress for Experimental Cytology, which was to have been held in Stockholm, Sweden, from July 25 to August 1, has been postponed indefinitely

The Illinois Society of Pathologists was organized on April 6, 1940, with J J Moore as president, O T Schultz as vice president and I Davidsohn as secretary-treasurer

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Pathologic Anatomy

TRAUMATIC AUTOTRANSPLANTATION OF SPLENIC TISSUE S JARCHO AND D H  
ANDERSON, *Am J Path* **15** 527, 1939

Two cases are presented in which a person who had undergone splenectomy for splenic rupture was subsequently found to have numerous nodules of spleenlike tissue scattered throughout the peritoneal cavity. Mention is made of 8 additional cases gathered from various sources and of 4 cases in which no definite history of trauma was available. In 2 of these the spleen and the left kidney were shrunk, scarred and distorted. Several analogous cases of a similar condition in an animal are cited from the literature. The experimental evidence is summarized, and it is shown that splenic tissue is susceptible to autoplasmic transplantation. The "splenoid" theory is described and is shown to be a gratuitous assumption, inadequately supported by evidence. It is concluded that the aforementioned nodules found in the peritoneum and omentum of persons who had undergone splenectomy for traumatic rupture of the spleen are due to autoplasmic transplantation of particles of spleen torn loose from the main body of splenic tissue and disseminated in part by the aid of hemorrhage.

FROM AUTHORS' SUMMARY

INTRACRANIAL ANEURYSMS C A McDONALD and M KORB, *Arch Neurol & Psychiat* **42** 298, 1939

McDonald and Korb tabulated 1,125 cases of, verified arterial aneurysm at the base of the brain and added 2 of their own. The earliest recorded cases date back to the middle of the eighteenth century, the latest, to 1937. Four hundred and seven contributions have been perused and arranged chronologically according to the year of publication. In 48 per cent of the cases the aneurysm was located at the internal carotid artery or at the middle cerebral artery, in 15 per cent, at the anterior communicating artery, and in 28 per cent, posterior to the internal carotid artery. The youngest patient was aged 1½ years, the oldest, 87. The pathologic changes present in 67.3 per cent were in the form of sclerosis of the arteries, mycotic emboli and syphilitic changes.

GEORGE B HASSIN

SQUAMOUS METAPLASIA, SIMULATING CARCINOMA, ASSOCIATED WITH PROSTATIC INFARCTION O S CULP, *Bull Johns Hopkins Hosp* **65** 239, 1939

Only 1 previous case of metaplasia in the prostate associated with infarction has been found recorded in the literature. Eight patients, presenting a total of 11 typical infarcts of the prostate, were studied, and in each one squamous epithelium was noted along the periphery of the infarct. In 5 of these patients the cellular changes had been interpreted previously as early carcinoma. The squamous cells in all were confined to the margins of the infarct, and the remainder of the prostatic tissue showed only typical benign glandular hyperplasia. Four of the patients are living and well as long as eight years after prostatectomy and have given no clinical or roentgen evidence of carcinoma. Three patients died from other causes, and no metastatic lesions were found at autopsy. From the evidence presented in this study it seems clear that squamous metaplasia simulating carcinoma frequently occurs along the margin of the prostatic infarct. This condition should not be confused with the very rare squamous cell carcinoma of the prostate.

FROM AUTHOR'S SUMMARY

LIVER NECROSIS FOLLOWING BURNS, SIMULATING THE LESIONS OF YELLOW FEVER  
T H BLT, J Path & Bact **48** 493, 1939

Four patients with extensive superficial burns who died within four days presented severe damage of the liver with midzonal necrosis Councilman lesions and intranuclear inclusion bodies These findings were practically indistinguishable from those occurring in yellow fever

FROM AUTHOR'S SUMMARY

EMBOLIC MYOCARDITIS S DI NAVASQUIZ, J Path & Bact **49** 33, 1939

Myocardial lesions, the characters of which are defined, were encountered in 19 of 20 cases of subacute bacterial endocarditis (95 per cent) These lesions developed as a result of embolism of the coronary arteries or arterioles, the emboli being fragments of vegetations which varied in size and bacterial content They were specific only so far as they showed evidence of embolism and polymorphonuclear cell reaction There was no demonstrable relationship to the degree of cardiac failure, the cardiac valve affected, the character of the vegetation or the duration of the disease

FROM AUTHOR'S SUMMARY

SILICOSIS OF THE SPIGON AND THE SILICOTIC NODULE T H BELT, J Path & Bact **49** 39, 1939

The silicotic nodule in its mature form has the following distinctive characters 1 There is a central focus of argentophil cellular remnants and dust 2 There is a redundant avascular collagenous capsule which contains little or no dust 3 There is a characteristic silicious ash after microincineration 4 Though there is no definite quantitative correlation between the silica and the lesion, the silica content of the tissue as a whole is in excess of the normal range

FROM AUTHOR'S SUMMARY

BASAL CELLS OF THE CERVIX UTERI R CARMICHAEL and B L JEFFERSON, J Path & Bact **49** 63, 1939

Small, poorly differentiated basal cells are present in patches, often exceeding 1 mm in diameter, in the endocervical epithelium of almost all human cervixes, they may occur either in the glands or at the surface of the canal and are usually more abundant in the upper half of the canal They are apparently an undifferentiated product of the primitive cervical lining and probably act as reserve depots from which regeneration can take place after extensive destruction of the ordinary mucus-forming epithelium

FROM AUTHORS' SUMMARY

MYOCARDIAL SCARS T E LOWE, J Path & Bact **49** 195, 1939

In a series of 5 cases the myocardial scars in the ventricles are reconstructed and described in detail These scars are shown to have an anatomic position consistent with their being portions of one of the various muscle groupings of the ventricles It is suggested that they represent the end stage of myocardial infarction due to interference with the blood supply to a portion of a muscle bundle, though this is not necessarily the result of coronary thrombosis or embolism

FROM AUTHOR'S SUMMARY

INTRAMESENTERIC DIVERTICULUM OF THE SMALL INTESTINE J MILLGREN, Virchows Arch f path Anat **302** 677, 1938

A 15 cm long intramesenteric diverticulum of the small intestine furnishes the basis of a thorough review of the literature relating to this anomaly and of a discussion of the theories advanced to explain the condition Characteristic of such diverticula are their intramesenteric situation, their relative length, their gross and microscopic similarity to the normal intestine and their blind ending

In the author's opinion, they are derived from the omphalomesenteric duct, but additional abnormality of development of the first part of the intestine must occur to account for the intramesenteric situation of the diverticulum

O T SCHULTZ

PARASITIC POSTERIOR REDUPLICATION OF THE HEAD H KAHRMANN, Virchows Arch f path Anat **302** 742, 1938

Kahrmann describes 2 examples of a rare monstrosity characterized by the presence in the midline of the back of a reduplicated fetal head. The condition is the result of a second and incomplete invagination at the time of gastrulation.

O T SCHULTZ

### Microbiology and Parasitology

GROWTH AND METABOLISM OF TUBERCLE BACILLI R R HENLEY and P W LEDUC, Am Rev Tuberc **40** 313, 1939

Studies were made of the growth, metabolism and tuberculin production of cultures of *Mycobacterium tuberculosis*, human type, PN strain, on mediums containing different proportions of glycerol and of nitrogen from different sources, chiefly asparagine, glycine and the ammonium salts of glutamic, malic and malonic acids. The production of acids was related to the proportion of glycerol present and to the source and proportion of nitrogen, but not to the amount of growth, nor to the rate of growth, nor to the amount of glycerol consumed. On mediums containing nitrogen at high levels cultures always became alkaline during the earlier stages of growth when the nitrogen was derived from asparagine, glycine, ammonium malate or ammonium glutamate. On malonate mediums under like conditions cultures first became acid, then, as the glycerol supply was depleted, the  $p_H$  values tended to rise. With the five sources of nitrogen mentioned, a definite relation was found between the glycerol-nitrogen ratio of the medium and the final reaction of the culture, the higher the ratio, the more acid the culture tended to become. With each of the five sources of nitrogen, the yield of bacteria obtained was dependent on, but not proportional to, the amounts of glycerol and nitrogen present. Yields approximating 2 Gm of bacteria, dry weight, in 100 cc of medium were obtained with each source. Under favorable conditions yields of 10 Gm of bacteria were obtained from each gram of nitrogen, but under no conditions did the yield exceed 0.38 Gm of bacteria per gram of glycerol. In mediums containing available nitrogen at high levels the glycerol supply was exhausted about the time the maximum weight was reached, at low levels glycerol continued to disappear after the bacteria stopped increasing in weight. The nitrogen metabolism in cultures on asparagine medium was studied. When they became acid, the fall in  $p_H$  value occurred about the time that the filtrate nitrogen was reduced to a minimum. No consistent relation was detected between the final  $p_H$  value and the residual ammonia in the filtrate. Potent tuberculins were obtained from cultures grown on mediums in which nitrogen was supplied by any of the five sources of nitrogen mentioned. The conclusion was reached that the nitrogenous constituents of the medium are the chief source of the alkali and that glycerol is the chief source of the acid, which is probably produced as an intermediate oxidation product and is in turn oxidized.

H J CORPER

EXPERIMENTAL TRANSMISSION OF POLIOMYELITIS TO THE EASTERN COTTON RAT, SIGMODON HISPIDUS C ARMSTRONG, Pub Health Rep **54** 1719, 1939

The first symptoms of poliomyelitis noted in the cotton rats were a roughened appearance of the fur and a tendency to react by violent jumping when agitated. Flaccid paralysis developed in all animals which were considered affected. The

legs were paralyzed in all combinations, and respiratory difficulty developed in several, with the respiratory rate falling as low as 30 per minute in some. Two rats with respiratory failure died, the others were etherized. A number of other rodents were inoculated with the virus utilized in this study, including groups of Swiss mice, in which successive transfers were made, but no positive results were secured in animals other than the cotton rats. The eastern cotton rat is not vicious, and it multiplies readily in captivity. It is hoped, therefore, that when a sufficient supply becomes available and the most susceptible age is determined, the cotton rat may prove to be a cheap, convenient and useful laboratory animal for the study of poliomyelitis. It is conceivable, however, that the results secured may be due to some peculiarities of this particular strain of virus.

FROM AUTHOR'S SUMMARY

INFECTIOUS POLYARTHRITIS OF RATS W. A. COLLIER, *J. Path. & Bact.* 48: 579, 1939

In *Rattus norvegicus* a spontaneous polyarthritis was observed, which was readily transmitted to white rats and field rats (*Rattus brevicaudatus*) by plantar, subcutaneous, intraperitoneal, intrapleural and intracerebral injections, characteristic polyarthritic symptoms ensued. For some rats the disease was fatal, others recovered, though in many of these the swelling of the joints persisted. All efforts to isolate a micro-organism of etiologic significance from the inflamed joints or from the organs have failed. On the other hand, bacteriologically sterile organs produced the disease on inoculation into other rats. The agent was found not only in the inflamed joints but in the blood, brain, lungs, pleural exudate, liver, spleen and peritoneal exudate. The agent was also present in animals in which no symptoms followed infection. Once the disease had been overcome there was distinct immunity to reinfection. Animals in which symptoms in the joints have not disappeared generally prove to be immune to subsequent inoculation, as do those which showed no signs of disease after the first injection. In no instance have complement-fixing, precipitating or neutralizing antibodies been discovered in the serum of immune rats.

FROM AUTHOR'S SUMMARY

TUBERCLE BACILLI IN PERSONS CLINICALLY NOT TUBERCULOUS A. SAENZ and G. CANETTI, *Ann. Inst. Pasteur* 62: 361, 1939

Organisms were found directly, with difficulty or not at all in tissues obtained at autopsy from 76 tuberculous adults who clinically were called nontuberculous. The tuberculin test on the inoculated guinea pig, periodically repeated, was a more sensitive indicator of infection than cultures. Some four fifths of the caseous or calcified lesions, as well as some fibrous lesions, appeared to be sterile. Sterilization seemed to be a function of histologic maturity, depending thus on the site infected and on the age of the subject, older subjects in whom the rapidity of healing was retarded showed less sterilization. Sixty per cent of the tracheo-bronchial lymph nodes examined showed organisms, whether or not they appeared normal. The studies suggest that persistence of hypersensitivity in the adult is due not so much to continued virulence of the lesions of primary infection as to successive reinfections.

FROM AUTHORS' SUMMARY

### Immunology

A SKIN TEST FOR DETECTING GROUP C HEMOLYTIC STREPTOCOCCIC INFECTION CAUSING EPIZOOTIC LYMPHADENITIS IN GUINEA-PIGS J. K. MOEN, *J. Exper. Med.* 64: 553, 1936

A skin test with a crude bacterial extract prepared from group C (Lancefield) hemolytic streptococci was used as a means of detecting possible carriers of the

streptococcus causing epizootic lymphadenitis in guinea-pigs. A positive reaction similar to a positive tuberculin reaction was considered presumptive evidence of present or recent infection with this streptococcus. In 330 supposedly normal guinea-pigs 20 positive reactors were found. Over a period of fifteen months 195 negatively reacting animals used as a breeding stock yielded 1,296 progeny, none of the breeding stock or their progeny showed evidence of spontaneous lymphadenitis. Skin tests of 100 of the progeny were all negative. The use of this skin test as a means of obtaining breeding stock free from the streptococcus causing spontaneous lymphadenitis is suggested.

FROM AUTHORS' SUMMARY

THE SPECIFIC POLYSACCHARIDES OF TYPES I, II AND III OF PNEUMOCOCCUS. H. HEIDELBERGER, F. E. KENDALL and H. W. SCHERR, J. Exper. Med. **64** 559, 1936

The thermolability of the specific polysaccharides of types I, II and III of *Pneumococcus* has been shown by three independent observations: (1) diminution of the viscosity of solutions on heating, (2) decrease in the amount of antibody precipitated from homologous rabbit antisera and (3) increased tendency (S III) to pass through a collodion membrane. These effects may be explained most simply as a partial depolymerization under the influence of heat. In air, particularly in the presence of broth, oxidation also appears to be involved. Improved and simpler methods of preparation based on these findings are given for A I, S II and S III. The resulting products precipitate more anti-S from homologous rabbit antisera than do the earlier preparations. The methyl glycoside of methyl galacturonate has been isolated from the hydrolytic products of A I and evidence of the ultimate structural unit obtained.

FROM AUTHORS' SUMMARY

HYPERSENSITIVENESS AND ANTIBODY FORMATION IN TUBERCULOUS RABBITS. J. FREUND, E. H. LAIDLAW and J. S. MANSFIELD, J. Exper. Med. **64** 573, 1936

Rabbits infected with bovine tubercle bacilli acquire hypersensitiveness to tuberculin, intracutaneously injected. This sensitiveness appears in the period from the second to the sixth week after infection and increases rapidly thereafter. Tests, as a rule, show fluctuation in the intensity of the sensitization. Sensitization is followed by an interval of several weeks preceding death during which the animals fail to react. Rabbits infected with bovine tubercle bacilli form antibodies that fix complement in the presence of tubercle bacilli. The antibodies appear after two weeks, increase during six to ten weeks and persist until the animals die. In the later period of infection the skin fails to react to tuberculin at a time when the serum contains complement-fixing antibodies.

FROM AUTHORS' SUMMARY

LIPIDS AND IMMUNOLOGIC REACTIONS. F. L. HORSFALL JR. and K. GOODNER, J. Exper. Med. **64** 583, 1936

Specific precipitates resulting from the interaction of type I antipneumococcus horse and rabbit sera and the homologous capsular polysaccharide have been analyzed by gasometric micromethods for total nitrogen, lipid nitrogen and lipid carbon. Lipid may, under certain conditions, form as much as 51 per cent or as little as 4 per cent by weight of the specific precipitates. The total lipid content of the specific precipitates, within the range studied, is entirely independent of the protein content. Lipid nitrogen forms a very small but detectable portion of the total nitrogen of the precipitates. The absolute lipid content of the precipitates is a function of the concentration of lipid in the reacting mixture prior to precipitation and seems to be governed by the laws of the phenomena of adsorption.

FROM AUTHORS' SUMMARY

BLOOD-GROUPING FACTORS IN HORSES V A HERMAN, J Immunol **31** 347, 1936

From a study of the blood of eighteen horses Herman concluded that there are four blood groups corresponding to those of man. This similarity was established by absorbing horse serums with human O cells, thus removing the species-specific agglutinins, and observing a group-specific agglutination of human blood groups A and B and of the corresponding blood groups in horses. Similar results were observed by treating human O serums with horse erythrocytes, containing the horse species-specific agglutinin O, and then testing the serums against both human and horse red cells. The iso-agglutinins in horse serum also resembled those of man. The group-specific properties were also found in certain organs of the horse. The distribution of the blood groups in 910 samples of horse blood was found to be: group O, 10 per cent, group A, 41 per cent, group B, 16 per cent, group AB, 28 per cent. Five per cent of the samples could not be classified.

I DAVIDSOHN

PRECIPITIN AND COMPLEMENT FIXATION REACTIONS OF POLYSACCHARIDE EXTRACTS OF BRUCELLA M HIGGINBOTHAM and L S HEATHMAN, J Infect Dis **59** 30, 1936

The results of the precipitin tests with the polysaccharide preparations from seven strains of *Brucella* seem to show that organisms otherwise classified as of the same type may possess variable antigenic properties. The precipitin test is not satisfactory for establishing the type of a strain of *Brucella*, although extracts from three of the seven strains were found to give a positive reaction with the homologous antiserum only.

The results support the contention that the use of a polyvalent antigen embracing some local strains should be included in the routine serologic examination for undulant fever. All the serums from human beings with brucellosis which showed agglutination with the stock antigens (*Brucella melitensis*, *Brucella abortus* and *Brucella suis*) gave positive precipitin reactions with one or more of the polysaccharides of *Brucella*. Although the series is small the polysaccharide precipitin test seems to have no advantage over the agglutination test as a routine procedure and has the disadvantage of being impracticable because of the time and cost of preparing extracts.

The results of complement fixation tests with the polysaccharide extracts of *Brucella* and specific antisera as well as of those with the extracts and patients' serums were not as clearcut as the precipitin reactions.

FROM AUTHORS' SUMMARY

COMPLEMENT FIXATION IN NORMAL SERUMS TREATED WITH NUCLEI OF RED CORPUSCLES OF CHICKENS AND WITH TISSUE EXTRACTS J F CHRISTENSEN, Ztschr f Immunitätsforsch u exper Therap **88** 325, 1936

In active serums of chickens complement was fixed with nuclei of the chickens' own red corpuscles and of the corpuscles of other chickens. While group-specific properties were noted in the whole erythrocytes, only species specificity was found in the nuclei. The reaction did not take place at 0 C and was absent when inactivated serum was used. Attempts to absorb the complement-fixing property with nuclear suspensions were unsuccessful in all but one serum. Inoculating chickens with nuclei and with red corpuscles did not increase the titers of the complement-fixing property of their serums. Immune serums produced in rabbits with the whole red corpuscles and with the nuclei of the red corpuscles of chickens brought out antigenic differences between the components of these cells. The active serums of chickens were later shown to fix complement with extracts of the chickens' own tissues and of those of different animals (duck, rabbit, sheep, calf and hog). The reaction with undamaged red cells of the rabbit and of the

sheep was very slight. Active serums of the sheep, horse and man were shown to possess the same ability to fix complement with different tissue extracts, and in the case of the serum of the rabbit inactivation at 56 C did not remove the complement-fixing property, but heating at 62 C destroyed it. The complement fixation with the normal serums is interpreted as a nonspecific "pseudoreaction". The necessity of a consideration of this phenomenon in complement fixation tests with active serums, and even with inactivated serum in the case of the rabbit, is obvious.

I DAVIDSOHN

VITAMINS AND IMMUNITY H J JUSATZ, *Ztschr f Immunitatsforsch u exper Therap* **88** 472, 1936

Rabbits that received food of sufficient calories but without vitamins showed a drop in the bactericidal properties of their blood and very poor precipitin response to injections of horse serum. Vitamin A did not improve the aforementioned properties. Small quantities of vitamin D raised the bactericidal properties of the blood but did not affect the antibody response, while large quantities influenced both properties adversely. The water-soluble vitamin B, in the form of yeast, had no influence on the bactericidal properties of the blood serum against staphylococci or on the formation of precipitin. Vitamin C, the natural and the synthetic product, as well as its sodium salt, effected a marked though transient increase of the bactericidal properties of the blood serum and enhanced greatly the production of precipitins, particularly if the vitamin was injected immediately preceding the injection of the horse serum or if it was mixed with it.

I DAVIDSOHN

## Tumors

OBSERVATIONS ON RATS TREATED WITH SEX HORMONES C S McEUEEN, *Am J Cancer* **36** 551, 1939

The occurrence is reported of 3 tar cancers of the skin, 1 uterine cancer and 1 gastric cancer in rats fed with an estrogen obtained from the urine of pregnant women. A tar cancer in 1 control rat is recorded. Whereas in rats treated with estrone (theelin) for long periods carcinoma of the genital tract and breast occurred, accompanied by other, usual estrogenic effects, with prolonged injection of testosterone the females went into permanent vaginal diestrus, the pituitary weights were not above normal limits, somatic growth was not inhibited and the predominant neoplastic lesions were fibrous tissue tumors in areas of injections, some of which became sarcomatous.

FROM AUTHOR'S SUMMARY

PULMONARY ASBESTOSIS K M LYNCH and W A SMITH, *Am J Cancer* **36** 567, 1939

During the past twelve years, in 2,343 consecutive necropsies Lynch and Smith encountered 7 cases of primary carcinoma of the lung, including 1 instance of this lesion in an early stage, discovered only on histologic examination, and 2 cases of asbestosis. Among these necropsies were 35 showing some degree of asbestosis, in the majority of instances this was a minor finding and not the cause of illness or death, in a few the condition was advanced. If all necropsies and all cases of cancer of the lung are included, the incidence of primary pulmonary carcinoma in this necropsy service over this period was 0.3 per cent. If the 35 cases in which asbestos deposits were shown and the 2 cases of cancer of the lung in association with asbestosis are omitted, the incidence was 0.21 per cent. Among the cases of asbestosis (35) the incidence of carcinoma of the lung (2 cases) was approximately 6 per cent. Whether this is to be taken as of significance, especially in comparison with the general rate, is questionable. The series of cases of asbestosis is small and the possible statistical error great. It has seemed desirable to record,



in addition, the observation that advanced asbestosis may lead to bronchial epithelial metaplasia of a type encountered in other locations where cylindric epithelium may give rise to squamous cell carcinoma

FROM AUTHORS' COMMENT

MYELOID LEUKEMIA AND NONMALIGNANT EXTRAMEDULLARY MYELOPOIESIS IN MICE W A BARNES and I E SISMAN, *Am J Cancer* **37** 1, 1939

Eight transmissible strains of myeloid leukemia that have been studied possessed characteristics which with rare exceptions remained unaltered in the course of successive subpassages. Transmission experiments indicated the neoplastic nature of these disturbances. The malignant cells in 5 cases were myelocytes and in 3 cases were myeloblasts maturing into promyelocytes or myelocytes. Although morphologically the individual cells of these leukemias with a single exception resembled normal cells, they differed from the latter as well as from one another in their behavior in inoculation experiments. These differences included ability to produce tumors, color of the leukemic infiltration, localization in various tissues and transmissibility to different stocks of mice. The myelocytes characteristic of one strain underwent mitotic division in tissue cultures and, like malignant cells, failed to mature. In 6 additional cases of myeloid leukemia attempted transmissions to mice that were not highly inbred were unsuccessful. The infiltrations in nonmalignant extramedullary myelopoiesis in mice may be as extensive as those in myeloid leukemia. Nonmalignant medullary myelopoiesis is frequent in apparently healthy old mice of one of the stocks studied. It often accompanies suppurative inflammations, particularly those of long standing, and spontaneous and transmitted neoplasms. The differentiation of nonmalignant extramedullary myelopoiesis from leukemia is occasionally difficult. Features of the nonmalignant disturbance include conspicuous maturation of myeloid cells, association with erythrocytogenesis, presence of megakaryocytes, absence of epicapsular and tumor-like infiltrations and failure of transmission to other mice. Parenteral administration of suspensions of *Bacillus coli* is a simple procedure to stimulate extramedullary hemopoiesis in mice. Exposure of mice with spontaneous mammary tumors to small doses of roentgen rays did not produce myeloid leukemia and failed to increase the extent of nonmalignant extramedullary myelopoiesis.

FROM AUTHORS' SUMMARY

INTRACEREBRAL CARCINOMATOUS METASTASES C C HARE and G A SCHWARZ, *Arch Int Med* **64** 542, 1939

Bronchogenic and mammary carcinoma commonly metastasize to the brain. In cases of cerebral metastasis the primary carcinoma is most commonly in the lung or the breast. In our series of 100 cases there were 65 in which the primary tumor was so located. A bronchogenic carcinoma often becomes manifest in the effects of a cerebral metastasis before there are any pulmonary signs. Carcinoma with cerebral metastases is not uncommon in persons less than 40 years of age. In our series it occurred in 27 of 100 such persons. The disease occurs predominantly in male patients, in the ratio 32:1, provided the cases of primary carcinoma of the breast are excluded. Symptoms of metastasis are usually of short duration before the patient becomes seriously ill. A gradual onset of intracranial symptoms in cases of metastatic intracerebral carcinoma, according to this study, is infrequent (17 per cent). The onset occurred suddenly in 36 of these 100 cases. Patients with metastatic cerebral tumor do not tolerate surgical procedures well. The average duration of life from the time of the first neurologic symptom until death was three and six-tenths months for the 32 persons who died while under the authors' care. The period of survival was much shorter for those who were operated on.

Signs of chronic debilitating disease may be absent. They were lacking in 40 per cent of the cases in this series. Abnormality of the spinal fluid is a

prominent finding. It occurred in 70 per cent of the cases in which the spinal fluid was examined. Roentgenographic erosion of the sella turcica may be present. In this series it was not uncommon, in spite of the supposedly short duration of the cerebral metastases. Encephalographic and clinical studies may localize one of the metastatic masses, which is usually the largest, and may fail to show the presence of other, smaller nodules. Even after metastasizing to the brain bronchogenic carcinoma may not appear as such on roentgenograms of the chest. Metastatic cerebral tumor may be single or multiple. Multiple tumor was observed in 20 brains removed at autopsy, single nodules, in the remaining 14. In practically all cases of cerebral metastasis there are metastases to other organs. Cerebral disease in addition to the metastases may be present. In 1 of the cases a cholesteatoma was observed in the cerebellopontile angle at autopsy, in 3 syphilis of the central nervous system was evidenced by a positive Wassermann reaction of the spinal fluid. Surgical removal of single metastases may in a few cases prolong life for months or even for several years, such cases, however, form a small percentage of those in which operation is done, most of the patients dying shortly after craniotomy. Subtemporal decompression often relieves the headache and affords great comfort to the patient and to his relatives. When craniotomy is to be performed, roentgen studies of the chest should be made regardless of the age of the patient.

FROM AUTHORS' SUMMARY

CARCINOMA OF THE BODY AND TAIL OF THE PANCREAS G L DUFF, Bull Johns Hopkins Hosp 65 69, 1939

An account of the pathologic features of carcinoma of the pancreas in general is followed by a description of the various modes and directions of the spread of cancer of the body or of the tail of the pancreas based on observations at autopsy in 16 cases. A comparison with the observations at autopsy in 16 consecutive cases of carcinoma of the head of the pancreas shows that primary cancer of the body or of the tail of the pancreas tends to spread much more widely and massively than carcinoma originating in the head. An adequate explanation of this tendency is afforded by the anatomic position and relations of the body and tail as contrasted with the head of the pancreas. Direct extension of carcinoma of the body or of the tail of the pancreas frequently leads to widespread involvement of the peritoneum and sometimes to invasion of the stomach or intestines. The abdominal lymph nodes are often massively involved. Invasion of the splenic vein with the consequent occurrence of massive metastasis to the liver may be followed by occlusion of the splenic vein, obstruction of large intrahepatic branches of the portal vein or even occlusion of the portal vein itself.

FROM AUTHOR'S SUMMARY

FROG CARCINOMA IN TISSUE CULTURE B LUCKE, J Exper Med 70 269, 1939

The adenocarcinoma of the leopard frog may be cultivated with ease in plasma mediums. In such cultures two types of growth occur with regularity. The first is in the form of tubules which promptly grow out in the solid medium and retain their tubular form as long as they remain completely enveloped by plasma. When, however, they make contact with the surface of the glass, they adhere to it, the part in contact becomes flat, and its cells now grow no longer as tubules but as membranes.

FROM AUTHOR'S SUMMARY

INHIBITION OF TRANSPLANTABLE TUMOR BY TISSUE EXTRACTS D A MACFADYEN, E STURM and J B MURPHY, J Exper Med 70 475, 1939

Mammary tissue of pregnant rabbits is found to contain an agent which inhibits growth of Bashford adenocarcinoma 63 in mice, but this material is without effect on Crocker sarcoma 180. Of the tissues so far studied, the mammary gland of the rabbit has yielded the most active product. From the results it

appears probable that pregnancy enhances the production of the agent, but this cannot be considered as established beyond doubt. The factor is found, with diminished activity, only in the protein fraction obtained from the aqueous extract by full saturation with ammonium sulfate (See also D. A. MacFadyen and J. B. Murphy [*J. Exper. Med.* **70** 461, 1939]).

FROM AUTHORS' SUMMARY

ISOLATION OF RABBIT PAPILLOMA VIRUS PROTEIN J. W. BEARD, W. R. BRYAN and R. W. G. WICKOFF, *J. Infect. Dis.* **65** 43, 1939

A protein free from carbohydrate and detectable by the Molisch test has been isolated by differential ultracentrifugation from extracts of infectious warts of cotton-tail rabbits. It gives a sediment with the sharp boundary characteristic of an undamaged molecular species and has a principal sedimentation constant  $s_{20} = \text{ca } 250 \times 10^{-13} \text{ cm sec}^{-1} \text{ dynes}^{-1}$ .

The yield of this protein was proportional to the activity of the virus in the extracts from which it was derived, varying from 1.0 mg per gram of very infectious warts to 0.008 mg per gram of warts of low infectivity. No protein has been obtained from noninfectious warts of domestic rabbits. The infectivity associated with the heavy protein was constant and was not related to the infectivity of the source extracts. Papillomas were consistently induced by the inoculation of  $10^{-9}$  Gm of protein.

Changes in the protein were accompanied by changes in the activity of the virus. Infectivity was destroyed when the protein was coagulated at 65 to 66 C or broken down by the action of acid or alkali. Virus infectivity was removed from the solution by precipitation of the protein at its isoelectric point and was associated with the precipitate under these conditions.

FROM AUTHORS' SUMMARY

CANCER OF THE THYROID GLAND J. DEJ. PRMBERTON, *Surg., Gynec. & Obst.* **69** 417, 1939

A series of 774 cases of cancer of the thyroid was reviewed, in 517 the diagnosis was established by microscopic examination, in the remainder the disease was obvious from clinical observation alone. The age incidence corresponds to that of carcinoma elsewhere, although the occurrence of this type of cancer in children is more common than is generally suspected. The sex incidence shows a ratio of 1 male to 174 females, whereas the ratio for benign nodular goiter is 1 to 5.07. In a large percentage of cases carcinoma of the thyroid originates in a benign adenoma, thus stressing the importance of prevention and treatment of lesions of the thyroid. The basal metabolic rate is inconstant and of no diagnostic aid in determining the presence of a malignant change in the thyroid. Sixty-eight per cent of the tumors were graded as 1 or 2 in regard to malignancy, based on anaplasia and dedifferentiation. Increased interest on the part of the pathologist is shown by the steady rise in the percentage of cancers discovered in patients operated on for tumors considered benign. The author classifies malignant neoplasms of the thyroid as papillary adenocarcinoma, adenocarcinoma in adenoma (malignant adenoma), diffuse adenocarcinoma and sarcoma. The distinguishing clinical features of papillary adenocarcinoma are the low grade of malignancy, marked radiosensitivity and tendency for the lesion to spread to regional lymph nodes, where it may be confined without further dissemination for many years. For these reasons radical removal and postoperative irradiation of the site offer a good chance of cure. Characteristic of adenocarcinoma in adenoma are the low grade of malignancy and tendency to early dissemination by way of the blood stream. Because of the lack of early involvement of lymph nodes and the invasion of blood vessels, the presence of cervical metastases of this type has a grave prognostic significance. Diffuse adenocarcinoma of the thyroid is commonly of higher grades of malignancy than the other types and behaves like such carcinoma elsewhere. Both squamous carcinoma and sarcoma of the thyroid are rare and very

malignant Metastases from thyroid carcinoma are most frequent in the cervical lymph nodes and next in the lungs The percentages of persons with malignant tumor of the thyroid who have lived three, five and ten years or more after treatment are 77, 70 and 58, respectively

FROM AUTHOR'S SUMMARY (WARRFN C HUNTER)

PAGET'S DISEASE OF THE NIPPLE R MUIR, J Path & Bact 49 299, 1939

The examination of 42 cases of Paget's disease of the nipple fully supports the view that the lesion of the nipple is due to intraepithelial growth of cancer cells which have spread from a malignant growth in the upper extremity of a lactiferous duct—intraduct carcinoma It also supports the view formerly expressed as to the association of Paget's disease with carcinoma of the mamma In all the cases intraduct carcinoma was present in the nipple Of 39 cases fully examined, it was present in both breast and nipple in 34, in 30 it was accompanied by infiltrating carcinoma of the breast The carcinoma of the breast which is so often associated with Paget's disease is ordinarily due to extension from an independent focus of intraduct carcinoma in the breast In view, however, of the mode of spread of intraduct carcinoma, the possibility of carcinoma of the breast resulting from a downward direct extension of intraduct carcinoma in the nipple must be admitted, but no undoubted example of this has been met with in this series of cases On the other hand, in 6 cases of carcinoma of the breast there was found an upward spread of intraduct carcinoma from a level below the nipple, and this had just reached, or was just reaching, the epidermis at the appearance of the clinical signs of Paget's disease The time of appearance or the stage of Paget's disease gives no information as to the presence or the absence of carcinoma in the breast The cases recorded here show a high frequency of carcinoma of the breast and emphasize the grave significance of the lesion of Paget's disease

FROM AUTHOR'S SUMMARY

SQUAMOUS CELL CARCINOMA OF THE TONSIL IN THE DOG F W WITHERS, J Path & Bact 49 429, 1939

Although references to tonsillar carcinoma in the dog are extremely rare in the literature, observations at the Royal Veterinary College, London, indicate a comparatively high incidence of the condition among town-kept dogs During the period of sixteen months covered by the cases under review approximately 20,000 dogs were presented for examination in the clinics of the college and, of those that died or were destroyed, 396 were examined post mortem Among these there were 100 with neoplasia, of which 53 had carcinoma of various kinds and, of the latter, 24 had tonsillar epithelioma The incidence is therefore approximately 12 per thousand of all dogs presented for examination, 6 per cent of all dogs examined post mortem and 24 per cent of all dogs with neoplasia There were 10 dogs with squamous carcinoma in sites other than tonsillar, and of these 8 showed the skin and 2 the urinary bladder affected Only 1 animal of a species other than the dog was recorded as having carcinoma of a tonsil during this period, namely, a cat, in which the primary lesion involved the tonsil, the soft palate and the root of the tongue, with a secondary deposit in the local lymph node, it was impossible to determine exactly the primary site of origin The condition appears to be commonest in dogs from 6 to 10 years of age, which is roughly the equivalent of 40 to 70 years in man

FROM AUTHOR'S DISCUSSION

EXPERIMENTAL EPITHELIOMA OF THE STOMACH A BESREDKA and L GROSS, Ann Inst Pasteur 62 253, 1939

The stomach of the rabbit shows pronounced receptivity to the Brown-Pearce epithelioma The appearance of the epithelioma in the stomach is preceded by a

short period of incubation. The tumor appears as a more or less voluminous growth localized at the point of inoculation, in about half the cases metastases occur in the internal organs. Rabbits with resorbed intracutaneous tumors of this type show solid immunity to inoculation of the Brown-Pearce epithelions in the stomach.

FROM AUTHORS' SUMMARY

ADENOMYOMA OF THE GALLBLADDER. P. EISENTH, *Virchows Arch f path Anat* **302** 717, 1938

Thirteen cases of adenoma, or better adenomyoma, of the gallbladder were encountered in a total of 4,000 necropsies. Of the two views as to the origin of adenomyomas—that it concerns congenital maldevelopment or that it results from chronic inflammation—the author accepts the former. A relationship between adenomyoma, chronic cholecystitis and cholelithiasis was not observed, although the cystically enlarged glands may become the site of intramural concretions. Transition of adenomyoma to adenocarcinoma was observed in 1 instance.

O. T. SCHULTZ

### Medicolegal Pathology

SUBARACHNOID HEMORRHAGE OF ANEURYSMAL ORIGIN. ITS INTEREST IN LEGAL MEDICINE. F. THOMAS, *Ann d'anat path* **13** 969, 1936

The article is a review of the subject of cerebral aneurysms. The literature, comprising 72 references, is well covered. A large number of photographs of gross specimens are included demonstrating many varieties of cerebral aneurysms and malformations of the basilar cerebral arteries. The etiologic varieties are discussed, i. e., arteriosclerotic, syphilitic, mycotic-embolic and congenital. The clinical factors relating to medicolegal problems are covered. In the discussion of congenital aneurysms emphasis is placed on the observation of the lesion in the younger age group and the absence of trauma or of antecedent disease.

PERRY J. MELNICK

CONGENITAL HEART DISEASE ASSOCIATED WITH SUDDEN DEATH. S. LYSS, *Deutsche Ztschr f d ges gerichtl Med* **31** 248, 1939

The cases discussed in this article were encountered in 20,000 postmortem examinations made at the Hafenkrankenhaus, Hamburg, from 1901 to 1938. The anomalies were grouped as (a) defects responsible for death, (b) defects incidental to death and (c) patent foramen ovale. The latter group was studied intensively only for the years 1936 and 1937. There were 11 cases in the first group, and in most of them septal defects and various distortions of the heart chambers were presented. When they occurred in children, they were noted during the first few hours or days of life, although their occurrence in stillborn infants was also observed. The only adult showing such an anomaly was a man 21 years old, who had pulmonary stenosis.

The second group included 22 persons with incidental cardiac and vascular defects which fell into the following categories: (a) ventricular septal defects, (b) atresia of the aortic isthmus, (c) aortic stenosis, (d) anomalies of valves and (e) patent ductus Botalli. This group contained a preponderance of adults except in the last division, in which there were 4 adults and 4 children.

Patent foramen ovale was found 78 times in 2,015 autopsies, an incidence of only 3.5 per cent. In 2 of the cases it was associated with paradoxical embolus. The low percentage is at variance with the observations of Aschoff, who placed the incidence between 20 and 30 per cent.

GEORGE J. RUKSTINAT

TRAUMATIC AITIRATION OF THE ELASTIC FIBROUS SYSTEM OF THE LUNG S  
OKROS, Deutsche Ztschr f d ges gerichtl Med **31** 308, 1939

Okros reported a comparative study of the elastic fiber system of the lung in the presence of a gunshot wound, a stab wound, a tear or compression made during life and post mortem. Somewhat similar changes took place in the elastic fibers with all of the aforementioned traumas when these were inflicted during life, but the changes were most marked after gunshot wounds. Characteristically, the fibers contract near the site of injury and form a dense, feltlike zone. At a distance of 6 to 15 alveoli removed from the traumatized zone the elastic fibers are torn, frayed or curled into ball-like contractures. All of these changes are lacking when the wounds have been made post mortem.

GEORGE J. RUKSTINAF

### Technical

MITOTIC LEUKOBLASTS IN THE PERIPHERAL BLOOD IN INFECTIOUS MONONUCLEOSIS  
H. BOWCOCK, Am J M Sc **198** 384, 1939

In infectious mononucleosis, mitotic leukoblasts and other unusual cell forms may appear in the peripheral blood near the peak of the leukocyte count. The occurrence of such immature and unusual cell forms is emphasized in order that confusion may be avoided in the diagnosis of this relatively benign illness, which often resembles acute leukemia in many respects. FROM AUTHOR'S SUMMARY

POLARIZED LIGHT FOR THE STUDY OF MYELIN DEGENERATION C. O. PRICKETT AND  
C. STEVENS, Am J Path **15** 241, 1939

A study has been made of the merits of the polarized light method as compared with the Marchi and sudan III methods of demonstrating myelin degeneration due to transection of the peripheral nerves in the rat. The polarized light method was found to be rapid and accurate. The changes depicted were consistent and did not depend for their demonstration on numerous technical manipulations. Changes in both myelin sheaths and axis-cylinders were visible in the same preparation. As compared with the polarized light method the Marchi method gave very inconsistent results. The sudan III method was consistent but failed to reveal the early changes following transection. Marked and advanced changes were shown by the polarized light method in nerves which had been transected only twenty-four hours. The earliest degeneration shown by the Marchi method was shown seventy-two hours after transection, and the earliest by the sudan III method, one hundred and twenty hours after transection. The thickness of the section influenced the structural detail observed by the polarized light method. Sections 10 microns in thickness showed more detail than sections which were thicker. It was found advantageous to uncross the analyzer prisms in determining the continuity of fibers which appear segmented, for distinguishing between edema of axis-cylinder and periaxillary accumulation of isotropic material and for revealing isotropic fibers masked by the crossed prisms. Sections of unfixed fresh normal or degenerating nerves when viewed by polarized light presented an appearance considerably different from that of fixed nerves. This does not detract, however, from the usefulness or reliability of the method.

FROM AUTHORS' SUMMARY

A MORE RAPID METHOD OF GUINEA PIG INOCULATION FOR THE DIAGNOSIS OF  
TUBERCULOSIS C. I. WOOLSEY, J Lab & Clin Med **24** 855, 1939

The average length of time required for the diagnosis of tuberculosis by intracutaneous injection of the exudates into guinea pigs is three weeks, in comparison with the six to eight weeks required by the formerly used subcutaneous route. Of the 173 guinea pigs used for injections in this series, only 5 died before the presence

or the absence of tubercle bacilli in the fluid could be demonstrated. Since the diagnosis is made from the initial lesion and substantiated by observation of regional adenopathy and by autopsy, there is little chance of a spontaneously infected guinea pig not being recognized as such. Those guinea pigs that remain uninfected for six weeks may be kept as breeding stock.

FROM AUTHOR'S SUMMARY

ARTIFICIAL AND SERUMLESS MAINTENANCE MEDIA L. C. BAKER and A. H. EBELING, J. Exper. Med. 69 365, 1939

Several mediums designed for maintaining the life of cells and of organs outside the body are described. Cultures made from a pure strain of fibroblasts have been maintained in these mediums in vital condition and with little or no proliferation for periods varying from forty-three to fifty-six days. One of these mediums is very simple, inexpensive and easy to prepare, and one is serumless.

FROM AUTHORS' SUMMARY

# Society Transactions

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## AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

ERNEST W GOODPASTURE, *President*

PAUL R CANNON, *Secretary*

*Twenty-Seventh Annual Meeting, New Orleans, March 13-17, 1940*

### **Preliminary Observations on Rabies in the Chick Embryo** JAMES R DAWSON JR, Vanderbilt University, Nashville, Tenn

Chick embryos have been infected by intracerebral inoculation of fixed virus, street virus and human virus of rabies, each of which produces typical Negri bodies throughout the nervous system of the embryo. One strain which has been maintained in chick embryos for forty-seven generations has exhibited profound alterations in its virulence for rabbits. Intracerebral inoculation of this chick embryo-adapted virus in rabbits is followed by development of clinical evidences of acute encephalitis in the form of fever, incoordination and weakness. Of 19 animals treated in this manner, however, 13 survived, following this mild self-limited disease they disclosed active immunity to the virus of rabies and were able to withstand an intracerebral injection of about 1,000 minimal lethal doses of either fixed virus or mouse brain street virus. Less dramatic alterations appear to have occurred in the pathogenicity of this strain for dogs and mice.

### **Sputum Studies as an Aid to Prognosis in Cases of Pneumonia** ARTHUR W FRISCH, Wayne University, Detroit

The number of extracellular encapsulated pneumococci per oil immersion field as determined from smears of rusty sputum treated with Wright's stain was significantly related to the incidence of bacteremia, leukopenia, multiple lobar involvement and mortality rate in 250 proved cases of pneumonia. Prognoses based on such counts were accurate enough to be of value in the selection of cases of potentially severe pneumonia both on admission of the patients to the hospital and during the course of therapy.

### **Immunochemistry of Catalase III Langmuir Multilayers with Serum Globulin** Lyman Fourt, University of Chicago

Using crystallized catalase as antigen and Langmuir's technics for the measurement of molecular dimensions, I have compared whole immune or normal serum and the corresponding globulins with respect to reaction in multilayers.

After an initial deposit has been made, repeated treatments with one substance produced little change. Starting from catalase, whole immune serum and its globulin fraction give nearly the same sequences of thickness changes: catalase, 50 angstrom units, anticatalase, 50 units, then catalase, 5 units, alternating with anticatalase, 50 units, to more than ten layers. An initial layer of serum is 25 units, while an initial layer of globulin is 70. Either the globulin molecules are differently oriented in each mode of adsorption, or the proportion of globulins varies, being least in the 25 unit deposit from whole serum. Anticatalase and catalase layers alternate from an initial layer of immune serum, but the first few



deposits are less thick than in the other series, some other systems fail to go on above the serum, although continuing well from the antigen

Specificity appears clearer cut with whole serums than with globulin fractions. Normal serum does not alternate with catalase, normal globulin deposits on catalase (25 angstrom units), but this nonspecific adsorption fails to continue. Serum globulin and whole serum of the same or different species alternate, resembling specific pairs.

#### Natural Quantitative Respiratory Contagion of Tuberculosis WILLIAM F. WELLS and MAX B. LURIE, University of Pennsylvania, Philadelphia

By means of the Wells air centrifuge, it is possible to determine quantitatively the number of tubercle bacilli suspended in a given volume of air by collecting the bacilli in a known volume of glycerin broth and seeding aliquot portions thereof on a modified Lowenstein egg medium. In an apparatus for the study of experimental air-borne disease rabbits were exposed to measured quantities of air-borne tubercle bacilli. The number of bacilli cultured from sample rabbits' lungs corresponded to the dose computed from the quantity of air breathed by these rabbits and the number of bacilli cultured from sample volumes of this air.

Rabbits which inhaled 1,000 or more tubercle bacilli died in a period of five to six weeks with massive nodular caseous pneumonia, without tuberculosis of any other organ, irrespective of whether they were naturally highly resistant or highly susceptible to tuberculosis. With smaller numbers of inhaled bacilli the natural resistance of the rabbit exposed played a determining role. When less than 100 units of bacilli was inhaled, the naturally susceptible rabbits died of massive caseous pneumonia with extensive caseation of the draining tracheo-bronchial lymph nodes and destructive lesions of hematogenous origin in the different organs—a picture of first infection resembling the childhood type of tuberculosis in man. Under the same conditions of exposure resistant animals developed a slowly progressive localized ulcerative pulmonary phthisis in which the draining lymph nodes and the internal organs were spared—a disease resembling the reinfection type of adult pulmonary tuberculosis in man.

The death of 96 per cent of tubercle bacilli suspended in the air was demonstrated when the air was exposed for three seconds to ultraviolet rays in the 2,500 to 3,700 angstrom wave band, as demonstrated by culture, inoculation in guinea pigs and inhalation by rabbits.

#### Comparison of Urinary and Serum Proteins WILLIAM A. MURRILL, M. H. SOULE and L. H. NEWBROUGH, University of Michigan, Ann Arbor

Urinary protein antiserum and total serum protein antiserum were prepared by injecting into rabbits the unaltered antigens from a patient with nephritis. By means of the precipitin reaction it was determined that these two types of antiserum reacted equally well with both homologous and heterologous antigens. Specimens of both types of antiserum were then adsorbed with total serum protein and the filtrates subsequently tested for the presence of specific urinary protein precipitins. The serum protein completely adsorbed all of the antibodies specific for both urinary and serum proteins, indicating that there are no antigenically active substances in the urine which are not present in the serum. In a like manner, samples of both types of antiserum were adsorbed with urinary protein and the filtrates tested for the presence of specific serum protein precipitins. The urinary protein antiserum gave negative reactions, whereas in the serum protein antiserum antibodies of high titer against total serum protein were demonstrated. The failure of urinary protein to adsorb completely the antibodies against total serum protein indicates that urinary protein is a fraction of total serum protein. These results support the classic view that serum protein is made up of two or more independent entities.

### Metamorphosis of the Nucleus of the Neuron in Inanition Due to Prolonged Starvation WARREN ANDREWS, Baylor University, Dallas, Texas

Several persons who died in an extremely emaciated condition showed marked degeneration of the nerve cells in the cerebral cortex with less striking changes in the cerebellum

Sections were stained by methods to be described. The cytoplasmic changes consisted of chromatolysis, vacuolation and final complete dissolution of the cell substance. The nuclear changes included migration of the nucleolus to an eccentric position (in the majority of cells) and great accumulation of basophilic granules in the nucleus, causing the nuclei to resemble those of the larger glial cells.

In all the cases extensive destruction of cells and neuronophagia were seen.

Experimental work on mice of different ages, which were starved for varying periods, has confirmed, in general, the conclusions as to the nature of the nerve cell changes brought about by inanition.

Many of the changes caused by inanition in mice and in men are similar to those occurring in senility. Age, therefore, is a factor which must be taken into account in studies of this type.

### The Pathologic Nature of Irradiation Sickness, A New Method for Inducing Shock VIRGIL H. MOON, KARL KORNBLUM and DAVID R. MORGAN, Jefferson Medical College, Philadelphia

Irradiation sickness was produced in dogs in order to make observations on the hemoconcentration and on the visceral changes. From 1,400 to 2,800 roentgens was given in divided doses over different parts of the abdomen. After an interval of sixty to seventy-two hours, severe illness developed, and hemoconcentration, ranging from 15 to 50 per cent, appeared. Urination was decreased, and blood was seen in the urine, feces and vomitus. The illness progressed rapidly, and death occurred by circulatory failure within twenty-four hours.

The gross and microscopic features were those characteristic of shock. They included capillovenous congestion of the viscera, petechial hemorrhages in mucous and serous surfaces, edema of the lungs and of the mucosae, and parenchymatous degeneration of the liver and kidneys. The results were concordant in 12 animals so treated.

An additional feature was degeneration, progressing to necrosis, of the gastrointestinal mucosa. The epithelium lining the crypts and covering the villi showed all stages of disintegration. Some crypts contained nuclear and cellular debris, others were denuded and empty. Some of the villi retained their epithelial covering while that in the crypts was necrotic and disintegrating.

Our results corroborate those of Whipple and others. Roentgen radiation of high voltage causes delayed necrosis of the intestinal mucosae, accompanied by shocklike manifestations. Apparently the latter are due to absorption of material from the damaged mucosa. The condition is accompanied by progressive hemoconcentration, and the visceral changes observed post mortem are characteristically those of shock.

These results provide experimenters with a new method for inducing shock by causing damage to the tissues. This method eliminates pain and emotional responses, sympathoadrenal hyperactivity, hemorrhage and anesthesia, conditions which are assigned as causes for shock by some writers.

### Effect of "Sex Hormones" on the Formation of Immune Bodies E. VON HAAM and IRENE ROSENFELD, Ohio State University, Columbus

In a series of experiments the influence of the administration of various doses of "sex hormones" on the development of agglutinating and protective antibodies against a laboratory strain of type I pneumococci was studied in rabbits. In

other experiments the mechanism of the protective action of these substances against lethal doses of the same organism was studied in mice. A certain quantitative correlation between the amount of such a substance injected and the amount of antibody formed could be demonstrated, and optimal immunizing or protective doses could be determined. Excessive doses of these substances proved definitely injurious to the development of antibodies. The role played by the interval between the administration of the substances and the production of the infection was investigated.

**Geographic Distribution of Poliomyelitis in Louisiana** ALBERT E. CASEY and BRANCH J. ALMOND, Louisiana State University and State Board of Health, New Orleans

Through the application of biometric procedures, especially of an objective method for detecting even the smallest epidemic, detailed and controlled studies on epidemic and sporadic poliomyelitis in Louisiana have been made possible. The relations of epidemic and sporadic poliomyelitis to geography, season, size of community, water supply, sewage systems and host factors, such as age, sex and race, are considered and a comparison with other diseases is made.

**Effect of Certain "Sex Hormones" on the Spread of India Ink and Resistance to Virus Infection** DOUGLAS H. SPRUNT and SARAH McDEARMAN, Duke University, Durham, N. C.

Last year we reported work which showed that certain endocrine states affect the spread of india ink in the rabbit's skin and also change the animal's resistance to infection with the virus of vaccinia. We have extended this work and can now report further studies. We find that the increased spread of india ink resulting from castration persists for a long period of time and that injected testosterone propionate has no effect on this spread. Injected estrogen, however, decreases the spread of india ink in castrates, male and female rabbits, and increases their resistance to infection with the virus of vaccinia.

**Effect of Temperature and of Starvation on the Character of Growth of Frog Carcinoma Implanted in the Anterior Chamber of the Eye** BALDWIN LUCKE and H. SCHLUMBERGER, University of Pennsylvania, Philadelphia

The adenocarcinoma which commonly occurs in the kidneys of leopard frogs is readily transplantable into the anterior chamber of the eye, where it develops according to well defined structural patterns. The manner and rate of such growth may be clearly observed over long periods through the slit lamp microscope and may be recorded objectively by photographs. This technic has been applied in the present study, which deals with the effect on tumor growth of two environmental factors, temperature and starvation. It was found that starvation of such a degree as led to great emaciation of the animals affected neither the manner nor the rate of growth. In groups of frogs maintained for months at two temperatures, 40° F. apart, the tumors grew somewhat more rapidly at the higher temperature, but there were no discernible effects of temperature on the manner of growth. It is concluded, therefore, that in a cold-blooded vertebrate, the frog, tumor growth is affected only to a minor degree by temperature and by starvation.

**Glycolipoid Antigen of Meningococcus and Gonococcus** ALDEN K. BOOR and C. PHILLIP MILLER, University of Chicago

Additional observations have been made on the factors influencing the demonstration of the glycolipoid antigen (Boivin) in the meningococcus and the gonococcus.

**Pathologic Changes in Experimental Gonococcic Infection** C PHILLIP MILLER and WALTER D HAWK, University of Chicago

The pathologic changes in the organs of mice intraperitoneally inoculated with gonococci suspended in mucin are described and compared with those resulting from the injection of sterile mucin alone

**Changes in the Distribution of Renal Phosphatase in Experimental Nephropathies** OPAL E HEPER, J P SIMMONDS and HELEN GURLEY, Northwestern University, Chicago

The phosphatase content of the kidneys of dogs was determined quantitatively by Bodansky's method, and its distribution in the renal units, by Gomori's micro-technical method. In normal dogs the phosphatase is distributed in a narrow zone along the free margin, i e, next the lumen, of the cells lining the proximal convoluted tubules. Poisons that induce degenerative changes in the epithelium of these tubules alter the distribution of phosphatase in the cells. The earliest change is a broadening of the band of phosphatase in the marginal portion of the cells. When actual necrosis has occurred, the phosphatase is diffused uniformly throughout the necrotic cell mass. It still stains, but less deeply, apparently not because of any material reduction in amount but rather because of wider distribution through the entire cell instead of along a narrow marginal zone. Chemical tests revealed little alteration in the quantity of phosphatase present in kidneys, even in those with extensive necrosis. The relation of this change to calcification in the renal tubules in experimental nephropathies is discussed.

**Virus of Lymphocytic Choriomeningitis in Man** MARION E HOWARD, Yale University, New Haven, Conn

Several strains of the virus of lymphocytic choriomeningitis have been isolated from the spinal fluid of patients presenting a clinical picture other than that of benign lymphocytic meningitis. There were 3 patients with encephalitis, 2 with meningoencephalitis and 1 who showed no evidence of encephalopathy. The virus was isolated as late as fifty-three days after the onset of acute symptoms, suggesting that the infection may be prolonged. The pathologic observations in 1 fatal case of encephalitis will be presented.

The strains isolated show some differences in their infectivity for different animal species, but all are immunologically related to the Rivers "W E" strain, as shown by studies of reinoculated immune animals. Up to the time of writing, neutralizing antibodies have been demonstrated in only 1 patient, and those in low titer forty-five weeks after the onset of acute illness.

**Anatomic Changes in Dogs Following Injections of Hematin** W A D ANDERSON, E F WILLIAMS and D B MORRISON, University of Tennessee, Memphis

Hematin in the form of disodium ferrihemate was injected into a series of dogs by subcutaneous, intravenous and intraperitoneal routes. At autopsy these dogs and their controls were examined by gross and microscopic methods. Widespread deposition of a brown pigment, which failed to give a prussian blue reaction, was found in mononuclear phagocytic cells and in cells of the reticuloendothelial system. Large amounts of the pigment were thus held within phagocytic cells and apparently remained innocuous. Vascular changes in the form of small hemorrhages and pigment thrombi occurred most frequently in the brain, endocardium and myocardium, particularly after intravenous administration. Striking lesions appeared in the kidneys. The most severe form was characterized by pronounced degenerative changes or massive necrosis of the convoluted tubules. Glomerular changes consisted of cellular proliferation, accumulation of polymorphonuclear leukocytes and formation of hyaline thrombi. Intraperitoneal admin-

istration of disodium ferrihemate over longer periods resulted in marked pigmentation of renal tubular cells and chronic changes in glomeruli. The changes produced by hematin are interesting in their similarity to the lesions of malignant tertian malaria and of hemoglobinuria from other causes.

#### Does Castration Alter the Incidence of Leukosis in Male Fowl? DAVID MARINE and SAMUEL H. ROSIN, Montefiore Hospital, New York

Thirteen instances of leukosis have occurred in 119 attempted castrations in white Leghorns. The leukosis was the diffuse hepatorenal type in 8, lymphosarcoma in 4 and a mixture of types in 1. The birds were obtained at the age of 5 to 6 weeks in five different batches, from three producers, and leukosis has developed in all batches. The youngest to die of the disease was 267 days old, and the oldest, 467. Fifty-three have been killed as slips. None of these slips showed any signs of leukosis, although of the same average age at death. None of the fowl with leukosis showed slitlike comb growth, although 2 had larger fragments of the left testis than some of the slips. All fowl with leukosis responded to androgens after they were known to have the disease. These data suggest that lack of testis hormones may have an activating effect on latent leukosis.

#### Production of the Thomsen Hemagglutination Phenomenon in Serum I. DAVIDSON and B. TOHARSKY, Mount Sinai Hospital, Chicago

From an O serum which was found to have the property of clumping O cells, a gram-positive bacillus was isolated which when inoculated into other serums, regardless of the blood group, causes those serums to agglutinate cells of all blood groups. Microscopically, it closely resembles members of the genus *Corynebacteria*.

When 52 individual serums, representing all the blood groups, and 10 pooled serums, each of the same blood group, were inoculated with the isolated bacillus, nonspecific agglutinins developed in 30 of the individual serums and in 9 of the pooled serums. The irregular agglutinins were demonstrated in undiluted serum and in diluted serum up to the dilution of 1:4. The agglutinins were active at 37 C. as well as at room temperature. On incubation at 37 C. and at 4 C., no change took place in serums inoculated with the bacterium.

Serums inoculated with the filtrate from a broth culture of the bacterium and incubated at room temperature contained nonspecific agglutinins at the end of four days. None incubated at 37 C. showed irregular agglutinins.

When the bacterium is inoculated into 2 per cent saline suspensions of cells of any blood group, it causes those cells to become panagglutinable by human serums. The same result is produced by the addition of proper amounts of a filtrate of a broth culture of the bacterium to suspensions of red blood cells.

#### Degeneration of Sensory Neurons in Pigs. MAXWELL M. WINTROBE, JOSEPH L. MILLER JR., HERMANN LISCO and LAWRENCE R. KOLB, Johns Hopkins Hospital, Baltimore

With the purpose of producing a condition similar to pernicious anemia, pigs weaned at an early age (3 weeks) were given a diet consisting of casein (25.8 per cent), sucrose (56.9 per cent), lard (10.8 per cent), cod liver oil (1.3 per cent) and a salt mixture, supplemented with brewers' yeast (3 Gm. per kilogram of body weight).

When they became accustomed to this artificial diet and seemed to be in a good nutritive state, the quantity of yeast given some of the animals was gradually reduced and thiamin, riboflavin, nicotinic acid and filtrate factor were given instead, separately and in various combinations. Ataxia developed in all of these animals, in spite of good growth in those receiving nicotinic acid. There was

degeneration in the posterior columns of the spinal cord, in the posterior root ganglions and in the peripheral nerves of the affected animals, in various degrees

In half the animals whose supply of yeast was not reduced, the ataxia and anatomic changes in the nervous system did not occur, and in the remainder the histologic changes were usually less extensive, although in several the ataxia seemed to be as great as in the pigs deprived of yeast. Wheat germ oil did not protect animals deprived of yeast. Observations to date indicate that a protective substance may be present in liver.

### Effect of Loss of Gastric Juice on the Electrolyte Balance of the Dog

CHARLES C. MACMAHON and STEPHEN MADDOCK, Boston City Hospital

Studies were made on dogs and pigs. A Heidenhain pouch was made in the fundic portion of the stomach, and the juice which was secreted was collected throughout each day. By returning this juice to the animals by stomach tube it was found possible to maintain them in an apparently normal condition for long periods. When the juice collected from the pouch was withdrawn and discarded, the well known characteristic clinical and chemical changes in the blood appeared. If the animals were given sodium chloride by mouth the gastric juice could be discarded without chemical changes occurring in the blood. When the sodium intake was kept up to normal by oral administration of sodium acetate, the animals remained in good clinical condition despite a fall in chloride ion to values as low as 65 milliequivalents per liter and a rise in carbon dioxide combining power to as high as 95 volumes per cent. With the sodium ion level maintained within normal limits (140 to 150 milliequivalents per liter), there was no significant elevation of nonprotein nitrogen or other signs of dehydration (oliguria, hemoconcentration, anorexia, weakness) despite the marked hypochloremia and alkalosis.

The maintenance of a normal sodium balance became difficult when the chloride levels fell below 60 milliequivalents per liter, and at levels as low as 40 to 45 milliequivalents per liter became impossible. Then the characteristic train of chemical and clinical changes developed. In animals deprived of the sodium as well as of the chloride ion these changes appeared when the chloride level approached 80 milliequivalents per liter. This relationship of rise in nonprotein nitrogen to fall in sodium ion in the electrolyte balance emphasizes the importance of the basic ion in maintaining a state of normal hydration.

### Effect of Complete and of Partial Biliary Obstruction on the Serum Phosphatase and Bile Acids of the Dog

STEPHEN MADDOCK, S. J. THANNHAUSER, VICTORIA MAGHEE and HAROLD REINSTEIN, Boston City Hospital and Boston Dispensary

We have stated previously that the ligation of one or more of the four hepatic ducts of the dog produced a transient rise in serum phosphatase, although jaundice did not supervene. Schiffman and Winkelman reported later that ligation of a single hepatic duct in each of 2 dogs led to an increase in phosphatase which persisted for thirty days. These authors apparently concluded that this was an invariable result. Our work has shown that some dogs fail to show such a rise following ligation of one hepatic duct, but that with ligation of two hepatic ducts all present a rise, which may be persistent. The ligation of the cystic duct can also produce marked elevation of serum phosphatase.

The serum phosphatase values in complete biliary obstruction in 40 dogs showed great variation in individual animals. In some the values did not rise much above 100 Bodansky units but in others the values rose as high as 460 Bodansky units eight weeks after ligation. Our experience has been that the values rise in a fairly smooth curve and gradually decline up to the time of death. This variation in the individual reactions of the animals led us to conclude that transfusion of normal blood to a jaundiced dog causes a rise in serum phosphatase. We now feel that such a statement must be made with some reservations until further work can be done.

In some of the animals bile acids have been studied by a recently published method of Jenke. This method has the advantage of giving a zero value for the blood of a normal animal. Following the ligation of the common duct there is a sharp rise in blood bile acids with a gradual decline after three or more weeks.

**Response of Different Strains of Rats to Nephrotoxin** JOSEPH E. SMADEL  
and HOMER F. SWIRT, Hospital of Rockefeller Institute for Medical Research,  
New York

Earlier studies on rats of the Whelan strain, in which severe nephritis had been induced with anti-rat-kidney serum, showed that in nephritic animals fed a high protein diet consistently a progressive disease developed from which they died within four to twelve months, whereas nephritic animals fed a low protein diet tended to recover from their acute nephritis. Administration of comparable amounts of nephrotoxin to rats of the Whelan and Evans strains resulted in acute renal injury of about equal severity. Wistar rats, on the other hand, displayed a milder degree of acute injury following the same dose of nephrotoxin. The acute nephritis regressed rapidly during the first month in Evans and Wistar rats maintained on a high protein diet. In the majority of these animals, however, recurrences developed after a latent period of two to six months. Although their urine became quite abnormal, only a few of these rats died of renal failure during the fourteen months of observation.

Differences in the response of the three strains of rats were dependent on hereditary factors and not on strain specificity of the nephrotoxin, since nephrotoxin prepared in rabbits by injecting kidney tissue from Whelan and from Wistar rats induced similar renal reactions.

**Production of Pressor Substance by Anaerobic Autolysis of Renal Cortex**  
JOSEPH VICTOR, ALFRED STEINER and DAVID M. WEEKS, New York City Hos-  
pital and Columbia University, New York

The experiments to be described were concerned with the role of oxygen in the elaboration of pressor substance by the kidney. Dog renal cortex was sliced about 0.2 mm. thick and incubated in 2 parts of plasma or Krebs solution at 37.5 C. in the presence and in the absence of oxygen for one to three days. The fluid filtered from the mixtures autolyzed in nitrogen showed on intravenous administration a powerful pressor action. The systolic blood pressure increased 20 to 300 mm. of mercury in 24 tests in 7 dogs. The fluid of the mixtures autolyzed in oxygen, on the contrary, decreased the blood pressure 40 to 186 mm. in 7 of 10 tests in 5 dogs. When the medulla of the kidney was prepared in a similar fashion, the fluid of the mixtures autolyzed in nitrogen increased the blood pressure only 4 to 25 mm. in 5 of 7 tests in 6 dogs. Medulla incubated in oxygen decreased blood pressure in 2 of 3 dogs.

Similar results occurred when sterile, cell-free saline extracts of beef or dog renal cortex or medulla were treated in the manner described. No pressor effects were obtained by comparable treatment of sterile, cell-free extracts of liver, spleen, lung or heart muscle.

**Effect of Induced Collateral Circulation from the Spleen to the Ischemic Kidney on Experimental Renal Hypertension** DAVID M. WEEKS,  
ALFRED STEINER, JAMES S. MANSFIELD and JOSEPH VICTOR, New York City  
Hospital and Columbia University, New York

The object of this investigation was to study (1) the influence on blood pressure of pexis of the spleen to the ischemic kidney, (2) the existence of collateral circulation in such preparations and (3) the effect on blood pressure of the subsequent removal of the joined organs. After a group of 8 dogs was maintained hypertensive by the Goldblatt method, an operation joining the cut parenchymas of spleen and kidney was performed on each. The blood pressure of all 8 dogs fell following this

operation Of the 7 animals showing a persistent fall in blood pressure, collateral circulation to the capillaries about the tubules, and not in the glomeruli, was demonstrated in 5 by injections of india ink and microscopic examination In the animals surviving the removal of the pexis more than three days, an elevation of blood pressure to the previous hypertensive level occurred A collateral circulation established by pexis of spleen and ischemic kidney increases the blood supply to the renal tubules and lowers the blood pressure in experimental hypertensive dogs

### Effect of Nitrogen Retention on the Regeneration of Plasma Proteins

RUSSELL L HOLMAN and J GILMER MEBANE, University of North Carolina, Chapel Hill

Experiments were performed to determine whether the impaired formation of plasma proteins in nephritis is related to retention of nitrogen or to other factors In normal adult dogs, maintained on a standard low protein diet, the plasma proteins, including the reserve stores were depleted to a basal level of 4 per cent by repeated bleeding and return of the washed red blood cells suspended in saline solution (plasmapheresis) After the hypoproteinemic state had been established, uranium nitrate was injected subcutaneously, and the effect of the ensuing elevation in nonprotein nitrogen on the production of plasma proteins was followed Three dogs have been tried, with uniformly negative results The data will be presented and their significance discussed

### The Antianemic Principle in Liver from Patients with Carcinoma of the Stomach and of the Cecum JOHN R SCHENKEN, JOSEPH STASNY and W KNOWLTON HALL, Louisiana State University and Charity Hospital of Louisiana, New Orleans

The administration of an extract prepared from the liver of a patient whose death was due to a scirrhus carcinoma of the pyloric and prepyloric portions of the stomach to 2 patients with typical addisonian pernicious anemia failed to produce a reticulocyte response The administration of a control extract prepared under exactly the same circumstances from the liver of a patient whose death was due to cerebral hemorrhage caused marked hemopoiesis in a patient with typical pernicious anemia

Extracts prepared from the livers of 2 patients who had died, the first with an extensive carcinoma of the stomach without involvement of the pylorus and the second with carcinoma of the cecum, stimulated hemopoiesis when injected into patients with macrocytic hyperchromic anemia

The antianemic principle in the liver was absent in the patient whose pyloric mucosa was replaced by neoplastic tissue and present in the liver of the patient whose entire stomach except the pylorus was involved by carcinoma It is of interest that the mucosa of the pyloric region, according to experimental observations, is the most active in the production of the "intrinsic factor"

### Effect of Varying Doses of Estrogen on the Incidence of Mammary Gland Carcinoma in Strain C<sub>3</sub>H Mice JOHN R SCHENKEN and EDWARD L BURNS, Louisiana State University, New Orleans

The relationship between carcinoma of the mammary gland and estrogen was tested quantitatively by injecting progynon B into 122 male mice of strain C<sub>3</sub>H, beginning at the age of 2 weeks These animals were divided into seven groups Group 1 received 3,000 rat units in two doses over a period of three days The remaining six groups received 100 rat units weekly for varying periods of time as follows group 2, four weeks, group 3, eight weeks, group 4, twelve weeks, group 5, sixteen weeks, group 6, twenty weeks, group 7, continuously throughout the lifetimes of the animals The controls were 28 untreated breeding males



and 46 untreated breeding females of strain C<sub>3</sub>H. Only animals which had lived five months or longer were included in this study. The incidence of tumors of the mammary glands was determined at necropsy.

No tumors developed in animals of groups 1 or 2. A low incidence of tumors was observed in animals of groups 3 and 4. A relatively high and essentially equal occurrence of tumors was noted in mice of groups 5, 6 and 7. No tumors were noted among the male controls. A slightly higher percentage of the female controls showed tumors than of any of the experimental groups.

#### **Effect of Intracutaneous Growth of Brown-Pearce Tumor on Preexisting Testicular Tumor** O. SAPHIR, M. APPFI and A. A. STRAUSS, Michael Reese Hospital, Chicago

It is well known that the transplantation of Brown-Pearce carcinoma into the testicle of a rabbit results in a large tumor with diffuse metastases. Spontaneous regression is rare. It is also recognized that the intracutaneous transplantation of Brown-Pearce carcinoma in a rabbit results in a cutaneous tumor which soon regresses, leaving the animal immune to subsequent transplantation of this tumor, regardless of the site of transplantation. These experiments brought forward an additional fact, namely, that in rabbits already afflicted with testicular Brown-Pearce carcinoma and metastases the intracutaneous transplantation of homologous tumor results in cutaneous tumors which often regress. Furthermore, following regression, the preexisting and coexisting testicular tumors and their metastases also undergo regression. The histologic features of these regressing tumors are discussed.

#### **Distribution of Large Doses of Radioactive Phosphorus in Rats** SHIELDS WARREN and R. F. COWING, Harvard University, Boston

Radioactive phosphorus, prepared by the department of physics of Harvard University by neutron bombardment of red phosphorus, was injected, in the form of dibasic sodium phosphate, intraperitoneally in rats. The dose given ranged from 16 to 30 microcuries per animal. The content in the blood dropped in the first forty-eight hours, and the content in the urine fell sharply also. Approximately 6 per cent of the amount injected was excreted in the first three hours. Measurements were made with a Lauritsen type electroscope. The experimental error is probably 15 per cent. The tissues containing most phosphorus were liver, voluntary muscle, bone, spleen and kidneys. The histologic observations will be reported.

#### **Induced Tumors of Salivary Glands of Mice** HAROLD L. STEWART, National Cancer Institute, Bethesda, Md

A solution of 1256-dibenzanthracene or methylcholanthrene in lard or light liquid petrolatum was injected into the salivary glands of 80 male and female mice of strains A and C<sub>3</sub>H. In 12 additional male mice of strain A cotton threads coated with crystalline benzpyrene were stitched into the glands, one in each, and allowed to remain. Tumors developed in 69 mice. Histologically, the tumors were squamous cell or adenosquamous cell carcinoma, spindle cell sarcoma, rhabdomyosarcoma or a mixed growth composed of one or more of the foregoing types. One tumor was unclassifiable. The average latent period for the development of the tumors in 19 male strain C<sub>3</sub>H mice and in 26 male strain A mice in which 0.34 mg of methylcholanthrene had been injected into the salivary glands was thirteen and one-tenth weeks and twelve and seven-tenths weeks, respectively. The possibility that the sarcomas may have originated from the muscular and connective tissues surrounding the salivary glands is under investigation.

**Radioactive Iodine as an Indicator in Thyroid Physiology Observations on Patients with Goiter** SAUL HERTZ, A ROBERTS, J H MEANS and R D EVANS, Massachusetts General Hospital, Boston

In a series of patients with goiters of various types it was found possible to trace iodine by means of radioactive "labeling" and to determine not only the uptake by the gland but the rate of excretion of the iodine from the body. As a result, certain inferences as to the possibility of therapeutic application of radioactive iodine can now be made.

**Coronary and Aortic Sclerosis, Periarteritis Nodosa, Chronic Nephritis and Hypertension as Sequelae to a Single Experimentally Produced Widespread Calcium Precipitation in the Rat** ARTHUR W HAM, University of Toronto, Ontario, Canada

Rats fed either (1) a single massive dose of activated ergosterol or (2) a diet rich in calcium, phosphorus and phosphoric acid for three weeks were found to have, soon after, widespread calcification of their vascular systems and kidneys. The initial calcification in the arteries was followed in both cases by intimal proliferation, which seemed to be of a reparative character. Several series of animals given a massive dose of activated ergosterol were allowed to live until they died of the complications arising from the original calcification. These animals seldom lived for a year, but most of them lived for more than six months. At autopsy they presented a picture of severe arterial and renal disease, complicated by hypertension. The original lesion in the kidney consisted of calcification of tubules in the loop of Henle, calcification in and about the glomerular tufts and calcification of glomerular arterioles, as well as calcification in some of the larger vessels. This original lesion was followed by glomerular fibrosis and cyst formation in blocked tubules. Hyalinization in glomeruli and in arteries became prominent later, as did periarteritis nodosa in the intestinal arteries. More than half the animals revealed the latter condition, which has been shown by others to be related to hypertension in the rat. None of the controls exhibited this lesion.

**Transformation of the Mycelial Form of *Histoplasma Capsulatum*, Darling, to the Yeastlike Form in Mice** ROBERT J PARSONS, University of Michigan, Ann Arbor

Recently *Histoplasma capsulatum* was isolated on biopsy of a nasal septal ulcer of a female patient. The granulomatous tissue about the ulcer contained great numbers of large mononuclear phagocytes which were heavily parasitized by yeastlike bodies, tentatively designated as *Histoplasma capsulatum*. Cultures of the ulcer base on tartaric acid-agar medium at room temperatures (Pellett) resulted in growth of the mycelial form of *H. capsulatum*. Since only the yeastlike form produces progressive disease in man and animals, I wished, for experimental purposes, to transform the mycelial to the yeastlike form. This was successfully accomplished by injecting the mycelial form intravenously into young mice. Autopsy of the mice has shown extensive parasitization of the so-called 'macrophage system of cells by the yeastlike form of the fungus.

**Genesis of Primary Pulmonary Lesions in Experimental Tuberculosis of Dogs** F D GUNN and MOORE A MILLS, Northwestern University, Chicago

The result of intrabronchial insufflation of adequate doses of virulent tubercle bacilli (human or bovine type) in dogs is at first a localized, low grade inflammatory reaction in the parenchyma, characterized by exudation of polymorphonuclear leukocytes and monocytes and by proliferation of septal cells. Cells of the large mononuclear exudate type predominate after about twenty-four hours. The lesions enlarge by direct extension in all directions and become solid in the center, and

necrosis begins in one or more foci, usually in several places at the same time in the larger pneumonic lesions. After caseation has become extensive, evacuation of necrotic material may occur if a bronchus of sufficient magnitude is involved. In this case, reaspiration of infectious material results in the formation of numerous foci of tuberculous bronchopneumonia in various parts of the lung. Otherwise, the mass becomes slowly encapsulated, calcium salts are deposited in the caseous matter, and after several months stainable acid-fast bacilli may disappear from the lesion.

Encapsulation of a lesion does not necessarily mean complete arrest of the inflammatory process, since the capsule tends to form just outside of the necrotic center and does not surround the entire granulomatous lesion.

Regional lymph nodes are involved within a few days after initiation of the parenchymal lesion. The degree of histiocytic hyperplasia and necrosis of lymphoid tissue depends on the number of bacilli reaching the node. Caseation of lymph nodes usually occurs in the presence of caseous parenchymal lesions. Calcification occurs after a few or several months in a small percentage of cases.

#### **A New Approach in the Therapy of Septicemia Due to *Streptococcus Viridans* in Experimental Animals** O. M. GRUHZIT, Parke, Davis and Company, Detroit

A study of substances of the sulfanilamide type in which the sulfur group had been substituted with carbon, oxygen, nitrogen, phosphorus, arsenic or their oxidation products resulted in the discovery of substances active against *Streptococcus viridans* in mice. Of these the paranitromethylbenzene and its oxidation product, the paranitrobenzoate, possessed marked effect, doses as low as 0.15 mg per mouse administered orally twice daily for three days prevented death in 80 per cent of mice whereas sulfanilamide gave negative results and sulfapyridine showed only slight activity. The paranitrobenzoate exerts a specific effect on *Str. viridans* in mice similar to that of sulfanilamide on the beta-hemolytic streptococcus. It possesses only slight activity on beta-hemolytic streptococci in mice and shows no activity against type I pneumococci or in tuberculous guinea pigs. The paranitrobenzoate is relatively nontoxic. Orally, for mice the minimal lethal dose is about 30 mg per mouse, and for rats it is about 2.5 Gm per kilogram; intravenously, the minimal lethal dose for rats is about 1.3 Gm per kilogram. Dogs receiving daily 50 to 200 mg per kilogram for two or three weeks have not shown anemia or cyanosis. Dogs have not shown nausea, vomiting or incoordination with doses of 300 mg per kilogram. Pigeons receiving 10 Gm per kilogram for seven days remained free from cyanosis or other complications. Experimentally, the administration of paranitrobenzoate appears to be a specific therapy for *Str. viridans* infection in mice.

#### **Influence of Diet on the Survival of Rats Repeatedly Exposed to Carbon Tetrachloride Vapor** JESSE L. BOLIMAN, The Mayo Clinic, Rochester, Minn.

Rats fed a diet adequate for moderate growth, which contained 40 per cent animal protein, 40 per cent carbohydrate and 8 per cent fat, were subjected to carbon tetrachloride vapor for thirty minutes three times each week. The average survival time of this group was thirty-one and three-tenths days. Other rats were fed one half of the control diet, the other half being replaced by an isocaloric equivalent of carbohydrate, protein or fat, respectively. Considering the survival time of the rats fed the control diet as 100 per cent, the survival time of the animals fed carbohydrate, protein or fat was 156 per cent, 105 per cent and 87 per cent, respectively. None of the treated animals consumed as much food as the untreated animals. The ratio of the food consumption was control diet 72, carbohydrate 82, protein 86 and fat 71. The animals of each group which consumed more than the average amount of food, however, did not survive longer than the other members of the group.

Marked necrosis of the liver was produced in all of the treated animals, and cirrhosis was extensive in all that survived a few weeks. Most of the animals died with massive hemorrhage into the small intestine. Such hemorrhages were not prevented by the administration of vitamin K by mouth. The histologic picture of the intestinal hemorrhages will be demonstrated.

#### **The Agent of Lymphogranuloma Venereum in the Fertile Hen's Egg**

GEOFFREY RAKE, CLARA M. MCKEE and MORRIS F. SHAFFER, Squibb Institute for Medical Research, New Brunswick, N. J.

In an investigation of the agent of lymphogranuloma venereum, with a view to studying the activity of certain chemotherapeutic drugs thereon, an initial difficulty was encountered in the low pathogenicity for experimental animals of the strains of lymphogranuloma venereum available. Use of Burnet's inoculation method in the fertile hen's egg did not produce much more satisfactory results. When, however, the yolk sac method of inoculation suggested by Cox was adopted, the virus was found to multiply very readily in the walls of this sac. Over twenty-five passages have been made by this method. Both infectivity and lethal titers of the agent for the developing chick through  $10^9$  have been obtained. Centrifugation of yolk sac preparations first at 3,000 and later at 10,000 revolutions per minute gives a sediment of small granules resembling morphologically the elementary bodies of the virus of vaccinia and similar to the granules described as specific for the agent of lymphogranuloma venereum by other workers. These granules appear to represent the infectious agent. They show a high infectivity compared to the supernatant obtained by supercentrifugation. The granules evoke specific antibodies in rabbits and chickens.

#### **Cultivation of the Virus of Lymphogranuloma Venereum on the Chorio-allantoic Membrane** MARION E. HOWARD, Yale University, New Haven, Conn.

The virus of lymphogranuloma venereum can be propagated on the chorio-allantoic membrane of the developing egg. Specific lesions were observed in only 30 per cent of the membranes, usually in those incubated four to six days. Histologically, these lesions resembled the small abscesses found early in infected human glands. Success of propagation is dependent on the time of transfer for a particular strain and the temperature of inoculation.

Egg membranes made into Frei antigen and tested in known cases elicited skin reactions varying in size. This variation seemed to be dependent on the presence or absence of lesions as well as on the time of incubation. Membranes which had been incubated from four to six days and which showed lesions usually gave the largest skin reactions. The infectivity of membranes seemed greatest after two to three days of incubation and was greatly reduced or absent after four to six days. Infectivity and antigenicity do not run parallel. This may explain some of the variations in human antigens.

#### **Effect of Extravasated Antibody on the Antigenicity of Extracts of Virus-Induced Rabbit Papillomas** JOHN G. KIDD, Rockefeller Institute for Medical Research, New York

The results of experiments will be given which show that large quantities of the rabbit papilloma virus are rendered completely nonantigenic on neutralization *in vitro* with the antiviral antibody and that extracts of the virus-induced growths of wild and domestic rabbits which contain much extravasated antibody may fail to stimulate the production of antibody even when massive amounts are injected repeatedly into normal rabbits. The fact was discovered incidentally that passively transferred antibody may be responsible for resistance to the papilloma virus following injections of extracts of the growths intraperitoneally into normal rabbits.

The findings disclose the strict limitations of immunization experiments of the sort described as a means of demonstrating whether "masked" virus is or is not present in extracts of the virus-induced growths. Since extravasated antibody is usually present in the growths, and since it not only neutralizes the virus when the growths are extracted but also renders the extract nonantigenic, it appears to be impossible to demonstrate by this method that "masked" virus is absent from a given extract unless it is known that antibody is also absent. The cancers deriving from the virus-induced papillomas of cottontail rabbits frequently contain an excess of extravasated antibody, and hence it follows that the attempted immunization of normal rabbits with extracts of these growths would fail to provide decisive evidence as to whether the virus is or is not present in them.

#### Lesions of the Tissues in Dehydration Shock HARRY A. DAVIS, Louisiana State University, New Orleans

Dehydration shock was produced in dogs by injecting subcutaneously 25 per cent sodium chloride solution in doses of 25 cc per kilogram of body weight. All of the animals presented evidences of extreme hemoconcentration, and death occurred within five to fifteen hours. The brain was cyanotic and revealed petechial hemorrhages in the leptomeninx and underlying cortical tissue. The capillaries were extremely distended, and the number of those visible was increased by actual count.

The lungs were reddish blue and studded with minute and larger hemorrhages. The capillaries in the alveolar walls were packed with red blood cells, and there was extravasation into the alveoli and beneath the visceral pleura. In those animals which survived for ten hours or more there were, in addition, focal areas of pulmonary edema. There were subendocardial and subepicardial hemorrhages, with petechiae between the myocardial fibers. The capillaries of the serosae and of the mucosae of the stomach, duodenum, jejunum and rectum were distended, so that these surfaces appeared dark red. The spleen revealed marked distention of the sinusoids and extensive hemorrhages beneath the capsule and about the malpighian corpuscles. The capillaries of the zona fasciculata and zona reticularis of the adrenal glands were markedly distended. In addition, there were petechial hemorrhages with minute areas of necrosis and diffuse and focal collections of polymorphonuclear leukocytes in this portion of the adrenal cortex. In the liver there was extreme distention of the capillaries about the central veins, as well as hemorrhages into the liver substance and beneath the capsule. The capillaries of the kidneys were distended, and there was marked albuminous change in the epithelial cells of the convoluted tubules. There was edema of the subcutaneous and intermuscular connective tissue at the site of injection, but no necrosis.

#### Chemical Analysis of Vaccinal Elementary Bodies CHARLES L. HOAGLAND, JOSEPH E. SMADEL and THOMAS M. RIVERS, Rockefeller Institute for Medical Research, New York

A chemical investigation of relatively pure preparations of elementary bodies of vaccinia, begun by Hughes, Parker and Rivers in 1935, has been extended to include quantitative determination of cholesterol, cholesterol esters, phospholipids, neutral fats, reducing sugars, amino acids, nitrogen, carbon and phosphorus. The analyses have also included similar determinations on the crude dermal pulp and on various discarded sediments from which the elementary bodies were finally separated. Whereas materials discarded early in the course of purification have been found to vary markedly in their chemical makeup from lot to lot (total fat, 80 to 100 per cent, nitrogen, 11.5 to 13.8 per cent, phosphorus, 0.3 to 0.48 per cent, reducing sugars after hydrolysis, 1.9 to 2.3 per cent), the final preparations of elementary bodies show a surprising constancy of analytic values (total fat, 40 to 55 per cent, nitrogen, 14.9 to 15.3 per cent, phosphorus, 0.57 to 0.59 per cent, reducing sugars, 2.5 to 3.0 per cent). Moreover, intradermal titrations of

each lot revealed a high degree of infectivity, consistent with the constancy of the analytic data. Such data tend to show a degree of chemical complexity not observed in certain plant viruses but approaching that of more highly organized bacteria and protozoa.

**Specific Neutralization of the Virus of Myxoma** ROBERT F. PARKER,  
Western Reserve University, Cleveland

Reliable results in the titration of the neutralizing potency of immune serum can be obtained when the "50 per cent" end point is used in titrating the virus. This end point, a theoretic dilution of virus which on inoculation should give rise to 50 per cent positive results, is calculated from the results of quadruplicate inoculation of tenfold dilutions of virus. The use of this or of a similar end point is necessitated by the particulate nature of the virus. Neutralizing capacity is then defined as the difference in titer of virus when it is titrated in the presence of normal and of a given dilution of immune serum.

By this test it has been found that serum of animals convalescent from infection with myxoma neutralizes small but constant amounts of virus and that the amount neutralized bears a regular relation to the concentration of serum. Serum of animals given injections of solutions of soluble specific substances but not active virus also neutralizes appreciable amounts of virus.

**Further Inoculation Experiments with the Virus of the Common Cold**  
H. M. POWELL, A. L. SPARKS and G. H. A. CLOWES, Eli Lilly and Company,  
Indianapolis

Six strains of the virus of the common cold following tissue culture propagation have been passed through Swiss mice, with scarcely more than very mild lesions developing. Virus alone did not kill, a few deaths occurred when organisms resembling Pfeiffer's bacillus were encountered as associated bacteria. Several passages of the virus through ferrets have been made, and frequent responses of temperature have been observed. We have observed little local effect when either attenuated or raw virus has been injected into human subjects. Raw virus when used in human subjects has appeared now and then to have been the cause of colds, so that further work is necessary before this agent can be used in man with success.

**Effect of Immune Serum and Vaccines on Experimental Arthritis Produced by the Filtrable Micro-Organisms of the Mouse Pleuropneumonia Group** ALBERT B. SABIN and ISABEL M. MORGAN, Rockefeller Institute for Medical Research, New York

The filtrable micro-organisms of the mouse pleuropneumonia group can be classified neither with bacteria nor with viruses. Since their pathogenic properties appear to depend on their invasion of and intracytoplasmic multiplication in certain mesenchymal cells, it was of interest to determine the effect of immune serum and vaccines before and after infection. Type B cultures intravenously injected into mice produced progressive, proliferative polyarthritis, with no evidence of involvement of any other organs or tissues. One cubic centimeter of immune rabbit serum (agglutinin titer, 1:2,000) injected intraperitoneally before injection of a culture completely prevented the arthritis in practically all mice; normal rabbit serum was without effect. When the immune serum was withheld until definite arthritis appeared, there was no obvious effect for a week or two, then the joints which had already become involved continued to become more severely diseased and even other joints became involved, after that time the arthritis in about 50 per cent of the mice cleared, while in the others it continued (in some to ankylosis) as in the controls. Vaccination with concentrated suspensions of micro-organisms killed by heating at 50 C for one-half hour did not have as good an effect as the

administration of immune serum, but qualitatively the results were of the same order. The type A micro-organism produces a different disease, which was affected by homologous immune serum in a similar manner.

#### **Role of Natural and of Acquired Immunity in Recovery from Virus Infections** ROBERT G. GREEN, University of Minnesota, Minneapolis

In some virus diseases recovery may depend more on the natural resistance of the host at the time of onset than on the acquired immunity arising during the course of the infection. The natural immunity seems to be due to a resistance of cells to invasion by the virus as well as to the presence of antiviral in the blood. The degree of susceptibility of the host is determined largely by the degree of susceptibility of those systems of cells to which the virus is adapted. In fox encephalitis recovery from the natural disease appears to be entirely dependent on the degree of natural immunity at the time of onset since fatalities occur regularly within the first five days of the disease and acquired immunity develops slowly. The injection of combinations of antiserum and live virus as vaccines results in some fatalities, which may be either early or delayed but are principally the latter. A slight degree of immunity acquired from a killed virus vaccine will protect against fatalities from serum and live virus combinations.

#### **Production of Immunity in Ferrets Against Various Strains of the Virus of Epidemic Influenza** FRANK L. HORSFALL JR and EDWIN H. LEVNETTE, the Laboratories of the International Health Division of the Rockefeller Foundation, New York

A vaccine containing both ferret spleen and lung has been found to be effective in producing immunity against different strains of the virus of epidemic influenza. The vaccine was prepared from tissues obtained from ferrets eleven days after intranasal inoculation of a 1939 strain of the virus and was inactivated by formaldehyde. A single subcutaneous injection of the vaccine resulted in rapid development of solid immunity against infection by strains of quite different antigenic structures. Vaccinated ferrets were found to be immune to both the PR8 and the W S strains as well as to various 1939 strains of the virus. The serum of vaccinated ferrets possessed the capacity of neutralizing large amounts of different strains of the virus within a few days after vaccination and thereafter.

Vaccines prepared in an identical manner from the tissues of ferrets which had been inoculated with other strains of the virus have failed consistently to produce immunity after subcutaneous inoculation.

## Book Reviews

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**Pathology** An Introduction to Medicine and Surgery J Henry Dible, Professor of Pathology in the University of London, and Thomas B Davie, Professor of Pathology in the University of Liverpool Pp 931, with 374 illustrations, including 8 plates in color Price, \$10 Philadelphia The Blakiston Company, 1940

The authors of this volume are experienced teachers of pathology with well defined views on methods of teaching The book is based on lectures which they have delivered to medical students This is evident from the agreeable informality, without loss of dignity or seriousness, that characterizes their presentation Pathologic changes are considered as a series of processes going on in the living body and leading to certain consequences—signs, symptoms, functional changes and morbid anatomic results In the text greater effort has been made to teach how the diseased conditions arise than how to recognize them once the morbid anatomic changes have become established The authors insist that the right places to learn to recognize morbid anatomic conditions are the postmortem room and the museum and that no amount of textbook teaching can supplant these This attitude explains some of the conspicuous features of the book

The conventional subdivisions, general pathology and special pathology, have not been followed As to the arrangement of their material the authors state that they have “fallen back upon that characteristically English solution of a difficulty a compromise” Inflammation, as the most fundamental process in a pathologic involvement, is considered first and then follow chapters on circulatory disturbances, degenerations, tumors and immunity This constitutes the first section of the book, covering 234 pages, with 119 illustrations The discussion of general principles and that of tumors (to which 79 pages are devoted) are briefer than is usually considered adequate for medical students in this country This brevity is made possible by emphasizing the dynamics of the various processes and by giving less attention to the more static features of gross and microscopic appearances

The second section of the book, 165 pages, deals with special infections This section begins with a detailed discussion of three illustrative types—of anthrax, as an example of a septicemic disease, of diphtheria, as a toxemia, and of actinomycosis, as a subacute inflammatory process This is followed by briefer consideration of common infections, classified according to their causative agents—staphylococcic, streptococcic, mycobacterial, spicular and anaerobic infections, rheumatism, gonorrhea, undulant fever, glanders, cholera and plague, and virus diseases (a chapter)

The third and final section, 397 pages, is given up to systemic diseases, and in it the more important pathologic conditions which have not already been dealt with are discussed Animal parasitic diseases are omitted entirely, likewise, gynecologic conditions In this third section a chapter on iron and pigment metabolism is placed between the chapters on the alimentary system and those on the genito-urinary system

Certain features of this volume deserve special mention The value of the descriptions of the “technique of blood examination,” pages 464-467, and of “tests for renal function,” pages 767-772, in a book of this kind is questionable because their necessary brevity renders them inadequate On the other hand, praise is due for the chapter on “Pathologic Changes in Respiratory Function” with special emphasis on Cheyne-Stokes respiration, hyperpnea, anoxemia and cyanosis, for the chapter on “Renal Function in Disease,” i e, the various types of nephritis, and for the table of “Findings in the Cerebrospinal Fluid” Among the numerous



tables and diagrams, most of which are pertinent and helpful, only a few can be mentioned. Figure 89 is a diagram of the spread of cancer from the primary growth by lymphatic permeation and embolism. In the diagrammatic figure 224 arrows indicate the direction of impedance to blood flow in cardiac decompensation due to left-sided valvular disease. Figure 276 illustrates the effect of emphysema of the chest on diaphragmatic respiration. Figure 305 shows the anastomoses between the portal and the systemic circulation. Figure 308 is a diagram of the production and relationship of the bile pigments. The schematic representation in figure 314, of the course and relationship of the different forms of nephritis, will be helpful. The effort to emphasize the clinical aspects of pathology is indicated by the inclusion of discussions of the chemotherapy of syphilis and of the serum treatment of pneumonia.

The subtitle, "An Introduction to Medicine and Surgery," reveals the purpose of the authors in preparing this book. It is doubtful if it will meet the requirements of American teachers of pathology as a textbook for medical students. But pathologists interested in teaching will find in it many useful methods of presenting material that is not new. The authors have made an effort, admittedly successful, to present the important facts of pathology from a dynamic rather than from a static point of view. To accomplish this they have omitted much that is considered indispensable in a standard textbook of pathology, but they have included much that usually finds no place in such a text. There has been a definite trend in this direction in the revised editions of textbooks on pathology during the past few years, but the present authors have gone beyond the writers of any of the more popular American texts. Because of the effective correlation between pathology and clinical medicine and surgery, this volume should be especially interesting and useful to practicing physicians. It will also meet adequately and effectively the requirements of a textbook in pathology for dental students because of its lack of unnecessary detail without undesirable abridgment of the descriptions of the more important pathologic processes.

In the selection of the 374 figures in the text the authors have intentionally "omitted many of those which illustrate common conditions which every student should be familiar with in the post-mortem room or the operating theater." The publishers have produced a book of excellent appearance. The type is large and easily readable, but with less important topics in smaller print. The illustrations are well reproduced, and their details are clear. Most of them leave little to be desired. The index occupies 34 pages and is well arranged, with the chief subjects announced in heavy type.

**The Rise of Embryology** Arthur William Meyer, Professor of Anatomy, Emeritus, Stanford University. Cloth. Pp 367, with 97 illustrations. Price, \$6. Stanford University, Calif. Stanford University Press, 1939.

In this volume Professor Meyer's historical talent finds its most ambitious expression. Some aspects of the subject matter have already been treated by him in independent essays, but most of the material is new. The method of approach is that of quotation from original sources, woven into a framework of explanation and comment. It is this presentation of obscure and widely scattered source material that makes the book especially valuable to readers who have neither the ability nor the patience to attempt the searching out, translating and collating of the original articles. Indeed, were ability and ambition equal to the task, access to the writings of many pioneers in biology would still be lacking to most.

Fifteen chapters treat of the following topics: early ideas of reproduction and generation, epigenesis, preformation, pangenesis, panspermia, search for the mammalian ovum, discovery, origin and meaning of the spermatozoon, changing ideas of impregnation, role of the "mule", problem of malformation, early visual aids, growth of morphology, early experimental embryology. There are 97 illustrations, 408 entries in the bibliography and an adequate index.

The serious worker in biology and medicine will welcome Professor Meyer's contribution with gratitude. It represents a laborious task done well by one eminently competent to select judiciously, to interpret penetratingly and to weigh with rare discrimination. Even immature students in these sciences should be directed to such representative chapters as that on the changing ideas of impregnation and that on the search for the mammalian ovum. Only in this way can the tyro be made to "realize that it required long and arduous effort, and clear thinking as well, to establish even the simplest facts—those he now takes for granted."

## Books Received

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CLINICAL ROENTGENOLOGY OF THE ALIMENTARY TRACT Jacob Buckstein, M D, Visiting Roentgenologist (Alimentary Tract Division), Bellevue Hospital, New York, Consultant in Gastroenterology, Central Islip Hospital Cloth Pp 652, with 525 illustrations Price \$10 Philadelphia and London W B Saunders Company, 1940

SHOCK AND RELATED CAPILLARY PHENOMENA Virgil H Moon, A B, M Sc, M D, Professor of Pathology, Jefferson Medical College, Director of Laboratories, Jefferson Medical College Hospital, Visiting Chief Pathologist, Philadelphia General Hospital Cloth Pp 442, with 30 illustrations Price \$3 50 London, New York and Toronto Oxford University Press, 1938

DERMATOLOGIC ALLERGY AN INTRODUCTION IN THE FORM OF A SERIES OF LECTURES Marion B Sulzberger, M D, Assistant Clinical Professor of Dermatology and Syphilology, Skin and Cancer Unit of the New York Post-Graduate Medical School and Hospital of Columbia University, Associate Attending in Dermatology and Syphilology, Montefiore Hospital, New York, Consultant in Dermatology and Syphilology, French Hospital, New York Cloth Pp 540, with 48 illustrations Price \$8 50 Springfield, Ill, and Baltimore, Md Charles C Thomas, Publisher, 1940

DIRECTORY OF MEDICAL SPECIALISTS CERTIFIED BY AMERICAN BOARDS 1939 Paul Titus, M D, Directing Editor Cloth Pp 1573 Price \$5 New York Columbia University Press, 1940

MEDDELELSER FRA DR F G GADES PATHOLOGISK-ANATOMISKE LABORATORIUM I BERGEN, 1939 Paper Various pagination Bergen, 1940

LA CREATINE ETUDE PHYSIO-PATHOLOGIQUE Jean Vague, Chef de clinique a la faculte de medecine de Marseille, and Jean Dunan, Chef de-laboratoire Preface by Professeur J Roche Paper Pp 256 Price \$1 40 Paris Masson & Cie, 1940

CHEMISTRY OF DISEASE Meyer Bodansky, Ph D, M D, Director of the John Sealy Memorial Laboratory and Professor of Pathological Chemistry, University of Texas School of Medicine Oscar Bodansky, Ph D, M D, Lecturer in Biochemistry, Graduate Division, Brooklyn College Cloth Pp 684 Price \$8 New York The Macmillan Company, 1940

## EXPERIMENTALLY INDUCED BENIGNANCY OF NEOPLASM

### IV SUPPRESSION OF MITOTIC ACTIVITY BY SO-CALLED IMMUNIZATION

AUSTIN M BRUES, M D

AND

WILLIAM T SALTER, M D

BOSTON

In studies conducted over twenty-five years ago on the inhibition of malignant growth produced by so-called immunization,<sup>1</sup> Woglom<sup>2</sup> had the impression that sarcoma so inhibited showed fewer mitoses in the histologic section than did the rapidly growing controls. He assumed that such momentary estimation of the number of mitoses measured the actual rate of the mitotic multiplication of cells going on in the tissue. This assumption was not checked by direct quantitative observations.

In recent years Dustin,<sup>3</sup> Allen and his co-workers<sup>4</sup> and Brues and Cohen<sup>5</sup> have introduced, in histologic studies, the use of colchicine to exaggerate the evidence of mitotic division. Although various investigators disagree at the moment on the mechanism involved, all agree that

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This investigation was aided by grants to William T. Salter from the International Cancer Research Foundation and from the Committee on Research in Endocrinology of the National Research Council.

From the Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.

1 Throughout this paper the terms "immunity" and "immunization" are used in a specialized sense to indicate resistance against tumor growth on the part of the host.

2 Woglom, W. H. The Nature of the Immune Reaction to Transplanted Cancer in the Rat, in Scientific Report on the Investigations of the Imperial Cancer Research Fund, London, 1912, vol. 5, p. 43.

3 Dustin, A. P. Sang **12** 677, 1938.

4 Allen, E., Smith, G. M., and Gardner, W. U. Am J Anat **61** 321, 1937.

5 Brues, A. M., and Cohen, A. Biochem J **30** 1363, 1936.

the results obtained with this drug reflect relative rates of cell multiplication or the "readiness of the cell to divide"<sup>6</sup> For this reason it seemed advisable to compare results obtained (a) directly, by determining the percentage of cells in mitosis in untreated tumors, with parallel results obtained (b) after preliminary treatment with colchicine

#### EXPERIMENTAL PROCEDURE

Pedigreed mice of the Bagg albino strain A were inoculated subcutaneously with sarcoma 180 These animals constituted the control group Test animals were first "immunized" by the procedure of Andervont<sup>7</sup> or by injections of nonviable tumor extract<sup>8</sup> The "immunized" animals were then inoculated subcutaneously in the groin with tissue the same as that used for the control animals Consequently there resulted in the "immunized" mice a graded series of tumors, so that these animals could be classified as having (a) no tumors, (b) small tumors and (c) larger tumors Indeed, a few of the tumors were very large On the contrary, all control animals usually had large tumors

At various intervals of time, ranging from fourteen to forty-five days after inoculation in the groin, test and control animals were put to death simultaneously The tumors were rapidly removed and fixed in Zenker's solution Paraffin sections of these tumors were prepared and stained with iron hematoxylin and eosin The tumors from the control animals are hereafter referred to as the controls, and the tumors from the "immunized" mice, as the "immunes" The mitoses were enumerated by the method previously described by Brues and Marble<sup>9</sup> Each result was recorded as the percentage of tumor cells undergoing mitosis in groups of at least 1,000 counted cells All stages of mitosis were included, from the disappearance of the nuclear membrane in prophase to the reappearance of normal nuclear configuration in telophase

*Colchicine*—Similar observations were made in parallel experiments with colchicine In preliminary tests a few mice were given varying doses of the alkaloid subcutaneously for the purpose of titrating the effect of the drug on mitosis by bioassay In this experiment the percentage of cells in each of the various stages of mitosis was determined and also the percentage of those in abnormal stages, i e, lacking spindles The results of this preliminary titration of the effect of colchicine are shown in table 1 They indicate that a dose of 0.025 mg in a 25 Gm mouse gave optimal exaggeration of mitotic activity Incidentally, this increase in mitoses was made up largely of abnormal forms Accordingly, 25 micrograms of colchicine was given subcutaneously to parallel series of control and "immune" adult mice from eight and one-half to nine hours prior to killing the animals

#### EXPERIMENTAL RESULTS

A comparison of the number of mitoses in control and in "immunized" tumors, both with and without preliminary colchicine treatment, is shown in table 2 These data constitute a complete experiment, designated as group A It can be seen that without colchicine mitoses are most numerous in controls during the first thirty days after inocula-

6 Dustin, A P Compt rend Assoc d anat, Basel, April 10-14, 1938

7 Andervont, H B Pub Health Rep **47** 1859, 1932, **49** 60, 1934

8 Salter, W T, and Wilson, H Unpublished data

9 Brues, A M, and Marble, B B J Exper Med **65** 15, 1937

TABLE 1—Percentage and Types of Mitotic Figures

		Percentage of Tumor Cells in Given Stage of Mitosis							
Colchicine Dose, Mg		Pro phase	Meta phase†	Ana phase	Tele phase	Abnormal Figures	All Stages		
Titration of colchicine effect *	0	0 15	0 50	0 35	0 05		1 05		
	0 010	0 20	1 00	0 30	0		1 50		
	0 025	0 10	1 20	0 30	0 10	4 50	6 20		
	0 060					1 10			
Distribution of mitoses by phases in tumors in group A	Controls treated with colchicine	0 2	0 2	0 15	0 15	1 5			
		0	0 7	0 2	0 2	1 1			
		0	0 4	0 4	0 2	5 7			
		0	0 1	0 1	0 2	3 6			
		0 1	0 8	0	0 1	13 5			
		0	0 7	0 2	0 1	7 8			
		0 1	0 5	0 2	0 1	4 3			
		0 06	0 49	0 18	0 15	5 90			
	Mean values								
	Mean values for 10 tumors not treated with colchicine								
	0 02	1 38	0 31	0 07	0 02				
	Immunes treated with colchicine	0 05	0 05	0 05	0 05	0 65			
		0	0 6	0	0 1	2 4			
		0	0 8	0 4	0	5 3			
		0	1 1	0 2	0 1	1 9			
		0	0 2	0 1	0	0 8			
		0	0 2	0 2	0	1 7			
		0	0 1	0 1	0 1	0 5			
		0	0 1	0 1	0	1 5			
		0 1	0 3	0 1	0 2	4 4			
		0 1	0	0	0	5 1			
		0	0 4	0	0	2 7			
		0	0 4	0 6	0 2	5 8			
		0	0	0 1	0 1	1 2			
		0 1	0 4	0 1	0 2	4 3			
	Mean values								
	0 02	0 25	0 13	0 07	2 45				
Mean percent- age of cells in mitosis by phases in tumors of group B	Time After Inocu- lation, Days		No in Series						
	Immunes	25	16	0 003	0 63	0 22	0 04	0 03	0 92
	Controls	25	29	0 03	0 93	0 42	0 06	0 02	1 46
	Controls	35	5	0 01	0 72	0 17	0 09	0 03	1 02

\* C57 mice bearing sarcoma 180 were used in this experiment Colchicine was given subcutaneously nine hours before the mice were killed

† The figures refer to cells in normal metaphase

TABLE 2—Statistical Analysis of Tumor Size and Mitotic Activity

Group A Percentage of cells in mitosis in controls and immunes with and without colchicine treatment	Tumors Treated with Colchicine		Tumors Not Treated with Colchicine			
	Controls 15-32 Day Tumors	Immunes 15-23 Day Tumors	15-29 Day Tumors	30-46 Day Tumors	13-25 Day Tumors	31-45 Day Tumors
	6 9	5 6	2 7	1 6	0 7	0 4
	4 0	3 9	2 8	1 4	2 3	1 2
	6 2	6 2	0 8	1 0	1 7	1 5
	12 8	3 2	1 2	2 1	1 4	1 4
	8 9	2 8	1 8	0 9	2 3	1 7
	10 0	0 9	3 3	1 5	2 4	2 7
	4 0	3 1	2 1	1 9	1 7	0 6
	2 2	6 5	2 7	0 8	1 2	2 2
	1 1*	13 3		0 8	1 4	2 5
	6 6	2 3		1 4	1 9	0 8
	4 0	1 1		1 0		1 9
	4 1	2 0		2 1		
	14 5	0 8		0 9		
	8 8	1 9		1 5		
	5 2	5 1		1 9		
		5 2				
		3 1				
		1 0				
		3 7				
		7 0				
		1 4				
		5 1				
Mean value	6 62	3 87	2 17	1 37	1 71	1 54
Standard deviation	3 53	0 60	0 83	0 45	0 54	0 73
Group B Mitotic activity and tumor size	Percentage of Cells in Mitosis		Length of Tumor Mm			
	Controls	Immunes	Controls	Immunes		
	Median	1 25	0 87	21 0	11 0	
	Mean	1 46	0 92	21 0	12 3	
	Standard deviation	0 53	0 47	5 6	5 4	
	Coefficient of variation	0 36	0 51	0 37	0 44	

\* This was a thirty-two day tumor

tion and that their number is decreased slightly in "immunes" at all intermediate intervals of time within this period. The older controls (i e., after thirty days) also have a low rate of mitosis.

After preliminary treatment with colchicine the same general relationships hold between controls and "immunes" as in the untreated series. The variability in all groups was rather large, as indicated by the standard deviations. This variability seemed due in large part to variability in the sizes of the respective tumors. There was striking consistency in the distribution of mitotic phases in the two groups, as seen in table 1, group A. In general, the averages of each phase in the control group showed twice the number of the "immune" group. Even more noticeable was the parallelism between mitosis and tumor

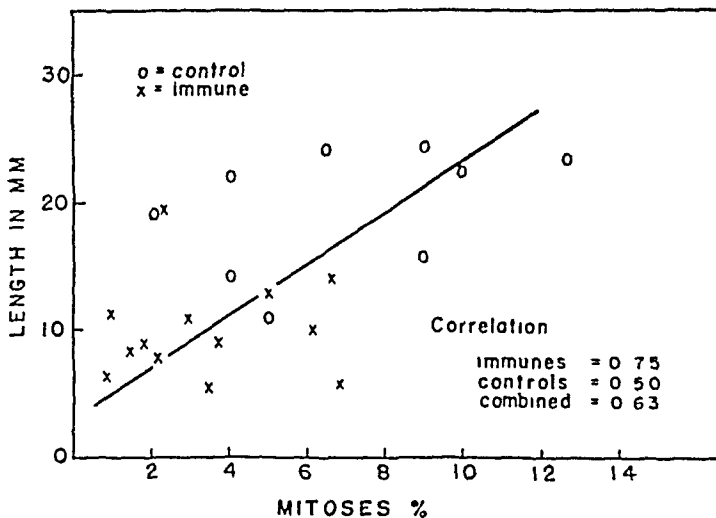


Fig 1—Relation between tumor size (expressed as length) and percentage of cells in mitosis ten hours after colchicine treatment and eighteen days after inoculation of mice with tumor. The coefficient of correlation for controls and "immunes" combined equals  $+0.63 \pm 0.09$ .

size. The values for a characteristic experiment with colchicine, in which all animals were put to death at the same time (eighteen days after inoculation with tumor), are shown in figure 1. It can be seen that there is good correlation between mitosis (after treatment with colchicine) and tumor diameter. It will be observed also that a single tumor in the "immune" group gave aberrant values for both size and mitoses, in short, "immunity" had failed to develop in this animal, possibly through lapse of technic. The coefficient of correlation between size of tumor and rate of mitosis for the mixed data (controls and "immunes" combined) is plus 0.63, for the "immunes" alone, plus 0.75, for the controls alone, plus 0.50.

In the tumors given preliminary colchicine treatment there is a great increase in the total mitosis count over that found in the untreated. This increase is obviously due to the presence of large numbers of abnormal figures, which may be classed as pseudometaphases in which the mitotic spindle is lacking. There are many dividing cells in which the spindle is present, however, and the late stages of mitosis are found in virtually all of these tumors. These cells, which show evidence of normal karyokinetic activity, are approximately half as numerous as in the tumors not treated with colchicine. This bears out an earlier observation on sarcoma 180<sup>10</sup> and suggests that in this tissue the abnormal figures represent only a prolongation of cell division.

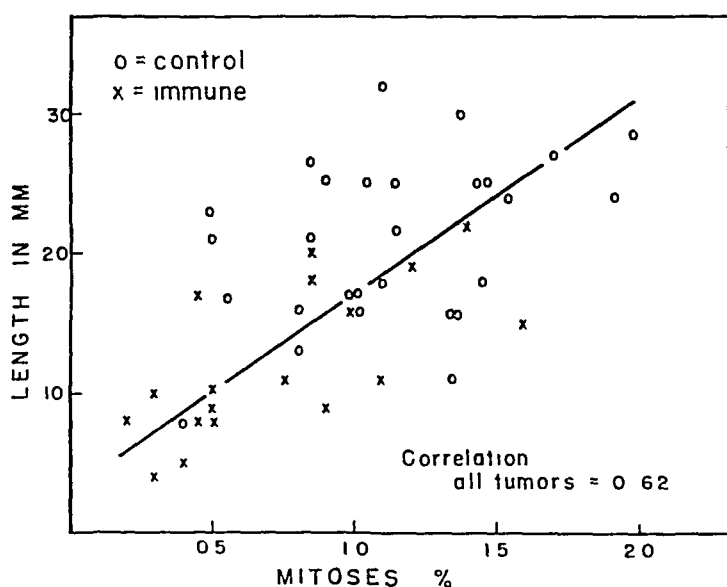


Fig 2—Relation between tumor size and percentage of cells in mitosis in tumors from mice not treated with colchicine twenty-five days after inoculation. The coefficient of correlation for controls and “immunes” combined equals  $+0.62 \pm 0.06$ .

A separated series of tumors, group B, was studied without colchicine treatment, as shown in table 1. For convenience, the data for “immunes” and controls are summarized in table 2, group B and in figure 2. Here, again, there was good correlation between size of tumor and rate of mitosis, i. e., 0.62 for the entire group of “immunes” and controls, combined. There was likewise good correlation in the distribution of mitotic phases in the two groups, as seen in table 2. The ratio between homologous values was only about 1.6, instead of 2.0, as in group A. Nevertheless, the same striking parallelism existed between mitosis and size in the two groups, as demonstrated by the



data shown. The ratio between mean values for controls and "immunes" is 1.7 with respect to length of tumor and 1.6 with respect to rate of mitosis.

#### COMMENT

These data show that during the period of active growth of tumors inoculated in mice, whether treated with colchicine or not, the more rapidly growing tissues exhibit a higher percentage of cells in mitosis. Consequently, the inhibition of growth produced by "immunization" actually does involve inhibition of the rate of cell division. Indeed, it appears that the "immune" and control tumors form a statistical continuum from small tumors with few mitoses to large tumors in which mitotic activity is great.

After a time the tumors inoculated in control animals assume the mitotic activity of young tumors growing in "immunized" animals. This phenomenon corresponds to the "concave growth curve" described by Schrek<sup>11</sup> and confirmed by Salter and Wilson.<sup>8</sup> This retardation presumably reflects the development of resistance to neoplasm occurring spontaneously in control animals, even though it is not sufficient to save the animals' lives.

The present figures suggest that about thirty days after inoculation with sarcoma 180 spontaneous "immunity" appears in the control animals, for the percentage of cells in mitosis drops to approximately that of the "immune" group. Although there is great variation in the percentage of cells in mitosis under all these various combinations of experimental variables, it seems considerably less than that recorded by Brues, Marble and Jackson<sup>12</sup> for regenerating liver. For this reason, it appears that a simple enumeration of mitoses in histologic sections of this tumor *without* preliminary colchicine treatment constitutes a good measure of the rate of cell multiplication. The colchicine technique is of greater value in the case of tissues in which the rate of mitosis is very low or fluctuates from hour to hour.

#### SUMMARY

A series of "immune" tumors (sarcoma 180 in strain A mice) shows a significantly lower mean mitotic rate than is seen in their controls. The mitotic rate is closely correlated with size in individual tumors, and in these characteristics immunized tumors and controls are shown to form a continuous series.

The effect of colchicine is to exaggerate the visible evidence of mitotic division. This exaggeration, as might be expected, is propor-

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<sup>11</sup> Schrek, R. Am J Path **12** 525, 1936.

<sup>12</sup> Brues, A. M., Marble, B. B., and Jackson, E. B. Am J Cancer **38** 159, 1940.

tionally higher in the group having a higher rate. It does not appear that the colchicine technic is necessary in the estimation of rates of mitosis in tumors.

The process of "immunity" to tumors involves retardation of the mitotic rate. This mitotic criterion of immunity appears in control tumors about thirty days after the mice have been inoculated, suggesting self immunity.

# ALVEOLAR LINING OF THE LUNG IN RELATION TO THE VIABILITY OF THE FETUS

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The presence or absence of a layer of epithelium lining the walls of the pulmonary alveoli has been the source of considerable controversy. For over eighty years, since the subject was first introduced by Thomas Addison such authorities as Chrzonszczowsky, Kolliker,<sup>1</sup> Eberth, Elenz, Colberg and, more recently, Lang, Rose,<sup>2</sup> Maximow and Bloom,<sup>3</sup> Clara,<sup>4</sup> Barnard and Day,<sup>5</sup> Miller<sup>6</sup> and Cooper<sup>7</sup> have reported conflicting results from histologic investigation of the alveolar lining. The controversy has narrowed down to the question of whether there is a continuous layer of epithelium, one made up of large non-nucleated plates interspersed with islands of small nucleated cells, or no alveolar cell lining at all but merely the walls of the capillaries surrounding the alveolar spaces. Miller<sup>6</sup> stated that the great obstacle to a correct understanding of the alveolar epithelium was the inability to dissect off the epithelial layer or to remove it by artificial means. He was able to overcome this difficulty by observing pathologic sections (e g, in cases of pneumonia) in which the epithelium was pushed off by the pouring of serous exudate behind it. Barnard and Day<sup>5</sup> investigated 48 human fetal lungs and came to the conclusion that although the early alveolar spaces were lined by pseudostratified epithelium, this lining began to disappear after the sixth month of embryonic life and did not reappear. Cooper<sup>1</sup> demonstrated by histologic sections that

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1 Kolliker, A. *Verhandl d phys-med Gesellsch in Wurzburg* **16** 1-24, 1881

2 Rose, S B. *Arch Path* **6** 36, 1928

3 Maximow, A, and Bloom, W. *Textbook of Histology*, ed 2, Philadelphia, W B Saunders Company, 1934

4 Clara, M. *Ztschr f mikr-anat Forsch* **40** 147, 1936

5 Barnard, W G, and Day, T D. *J Path & Bact* **45** 67, 1937

6 Miller, W S, in Cowdry, E V. *Special Cytology*, ed 2, New York, Paul B Hoeber, Inc, 1932, The Lung, Springfield, Ill, Charles C Thomas, Publisher, 1937

7 Cooper, E R A. *J Path & Bact* **47** 105, 1938

there was a continuous alveolar epithelium which became somewhat attenuated in later fetal life

Although the literature has presented voluminous discussions of the histologic nature of the alveolar lining, little attention has been given to the relationship between the type of cells lining the alveoli and the viability of the fetus. It is agreed that, whether the origin is ectodermal or mesodermal, large cuboidal cells make up the lining of the alveoli of young embryos and that after five or six months of intrauterine life these cells become flattened. The exact details of the supposed metamorphosis of the cuboidal "epithelium" into flat plates interspersed with small inconspicuous cells of the alveolar walls have not been thoroughly described. Some have claimed that this transformation occurs in the later embryonic stages by disintegration of the nuclei, degeneration of the cytoplasm and casting off of the cells. Others have stated that the change is accomplished by an encroachment of capillaries and desquamation of the "epithelium."

Whatever the origin of the cells and the nature of their transformation, the following questions arise. Are these large cuboidal cells compatible with life? Do they persist after five or six months of embryonic life as a pathologic phase of an earlier physiologic process? What relationship have they to livebirth and stillbirth of infants? Does the transition from nonrespiratory functioning cuboidal cells to respiratory functioning flat cells coincide with the transition from a previable to a viable infant?

The cause of stillbirths and neonatal deaths with no definite pathologic evidence of disease or injury has puzzled obstetricians, pediatricians and pathologists for many years, particularly when infants of viable age were born of healthy parents and by normal, uncomplicated deliveries. Potter,<sup>8</sup> in a recent survey of a large number of fetal deaths, showed that in only 57 per cent of the cases could a definite pathologic state be determined at autopsy. Public health statistics substantiate this observation—the difficulty encountered in establishing the cause of death during the neonatal period. In many instances the autopsy revealed only pulmonary atelectasis, which is not regarded as a primary cause of death but is interpreted as a secondary factor. Prematurity alone could not always be the cause of death, because some infants weighing 1,000 Gm survived, while many of those at term did not. Histologically, if one could demonstrate persistence of immature alveolar cells in a viable infant, the cause of death might be partially clarified.

Because of these problems, it was decided to study the lungs of human fetuses to determine the chronologic intrauterine age at which

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8 Potter, E. L. J. A. M. A. **112** 1549, 1939

the transition from cuboidal to flat cells occurs and to determine the relation between maturity of the alveolar cell lining and viability of the fetus

#### MATERIAL

The lungs of 50 newborn infants were examined grossly and microscopically. These lungs were obtained from liveborn and stillborn infants. The infants were subdivided, according to Scammon's classification (based on weight in grams, crown-heel length in centimeters and estimated intrauterine age), into abortive, previable premature, viable premature, full term and postmature groups.

#### METHODS

*Clinical Study*—In order to establish a complete clinical background for each specimen studied histologically, data on the following points were recorded for each infant: weight, height, sex, color, mother's last menstrual period, expected date of delivery, actual date of delivery, estimated intrauterine age, condition of infant at birth, signs of maturity or immaturity at birth, age of infant at death, mother's serologic record, and hours the infant was dead before autopsy was performed.

*Gross Pathologic Examination*—The weight, the crown-heel and crown-rump height, and the external evidence of maturity were noted. The position of the lungs in the thoracic cavity was observed. The color and consistency of the lungs were recorded. The lungs were tested to see if they would float in water, the individual lobes were also tested in this manner. The cut section was studied. The trachea and bronchi were examined for patency or occlusion by mucus.

*Fixation and Staining of Tissues*—Four pieces of tissue were fixed in each case studied, these included a section from (a) the apex of the right lung, (b) the hilus of the upper lobe of the left lung, (c) the hilus of the lower lobe of the right lung, and (d) the base of the lower lobe of the left lung. The tissue from the right lung was fixed in Schaffer's<sup>8a</sup> fluid, and that from the left lung was fixed in Carnoy solution<sup>8a</sup>. After fixation, the tissues were embedded and blocked in the usual manner.

The sections were stained routinely with hematoxylin and eosin. In some cases, additional sections were treated with elastin H, azan and fibrin stains.

*Microscopic Examination*—The state of expansion or collapse of the alveoli was noted. The cells lining the alveolar spaces were studied in great detail as to whether they were cuboidal or flattened or showed a combination of these types. The contents of the alveoli and bronchi were noted. Attention was also given to any unusual features, such as atypical cells or evidence of aspirated material.

#### RESULTS

*Age and Classification of Infants*—The lungs of 50 newborn infants were studied. Of these infants, 20 were born alive and 30 were stillborn.

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<sup>8a</sup> The composition of the fixing fluids is as follows: (a) Schaffer's fluid—2 parts of 80 per cent alcohol and 1 part of 40 per cent solution of formaldehyde, (b) Carnoy solution—6 parts of absolute alcohol, 3 parts of chloroform and 1 part of glacial acetic acid.

The duration of life in the liveborn was from a few minutes to forty-five days. With the exception of 2 infants (one living forty-five days and the other fifteen days) none lived for more than twenty-seven hours.

The intrauterine age as estimated from the time of the last menstrual period varied from 12 4 weeks to 44 7 weeks. According to Scammon's classification, 14 infants were in the abortive group, 12 in the previable premature group, 18 in the viable premature group, 5 in the full term group and 1 in the postmature group.

*Type of Alveolar Lining Cells Found*—Large, immature, cuboidal cells were found lining the alveoli of the lungs in 20 cases (figs 1 and 2). In 15 cases there was a mixture of cuboidal and flat, mature cells lining

*Relation Between Intrauterine Age and Type of Cells Lining the Alveoli of the Lungs*

Intrauterine Age, Weeks	Type of Cells		
	Cuboid	Cuboid and Flat	Flat
12 to 16 (4 cases)	4		
16 to 20 (5 cases)	4	1	
20 to 24 (10 cases)	8	2	
24 to 28 (11 cases)	3	7	1
28 to 33 (10 cases)	1	4	5
33 to 37 (4 cases)		1	3
37 to 45 (6 cases)			6

the alveoli (fig 3). In the remaining 15 cases there was a complete flattened alveolar lining such as that seen in the adult lung.

*Type of Cells Compared with Intrauterine Age*—A cuboidal, immature, nonrespiratory functioning type of cell was found lining the alveoli of all the fetuses under 16 weeks of intrauterine age and of 80 per cent of those under 24 weeks of intrauterine age.

A flattened, mature, respiratory functioning type of cell was found lining the alveoli of all infants that were over 37 weeks of gestational age and of 75 per cent of those over 33 weeks of gestational age.

A mixture of cuboidal and flattened cells was noted most frequently between the twenty-fourth and twenty-eighth week and almost as frequently between the twenty-eighth and thirty-third week of intrauterine age (table).

*Type of Cells According to Scammon's Classification*—Computations based on estimated intrauterine age are always subject to error, and it was deemed advisable to compare the type of alveolar cells with

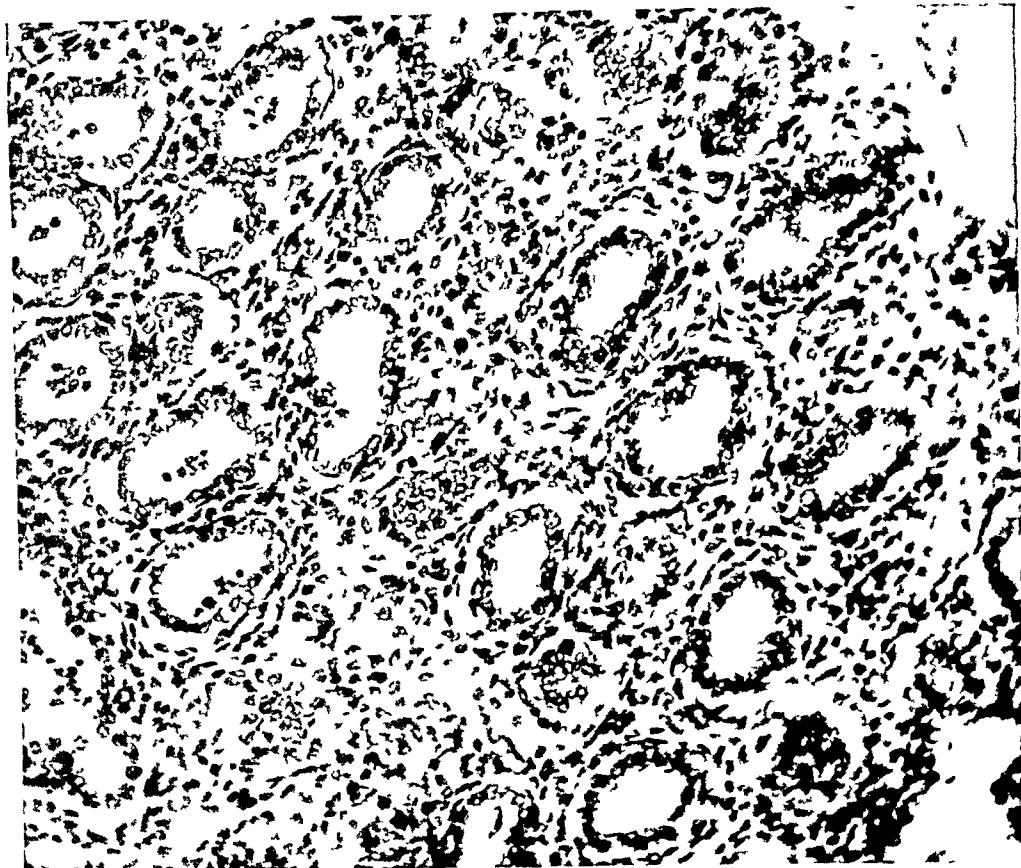


Fig 1—Lung of a stillborn infant (weight, 400 Gm , length, 27 cm , intra-uterine age, 21 weeks) of the abortive group Cuboidal cells form the lining of all the alveoli

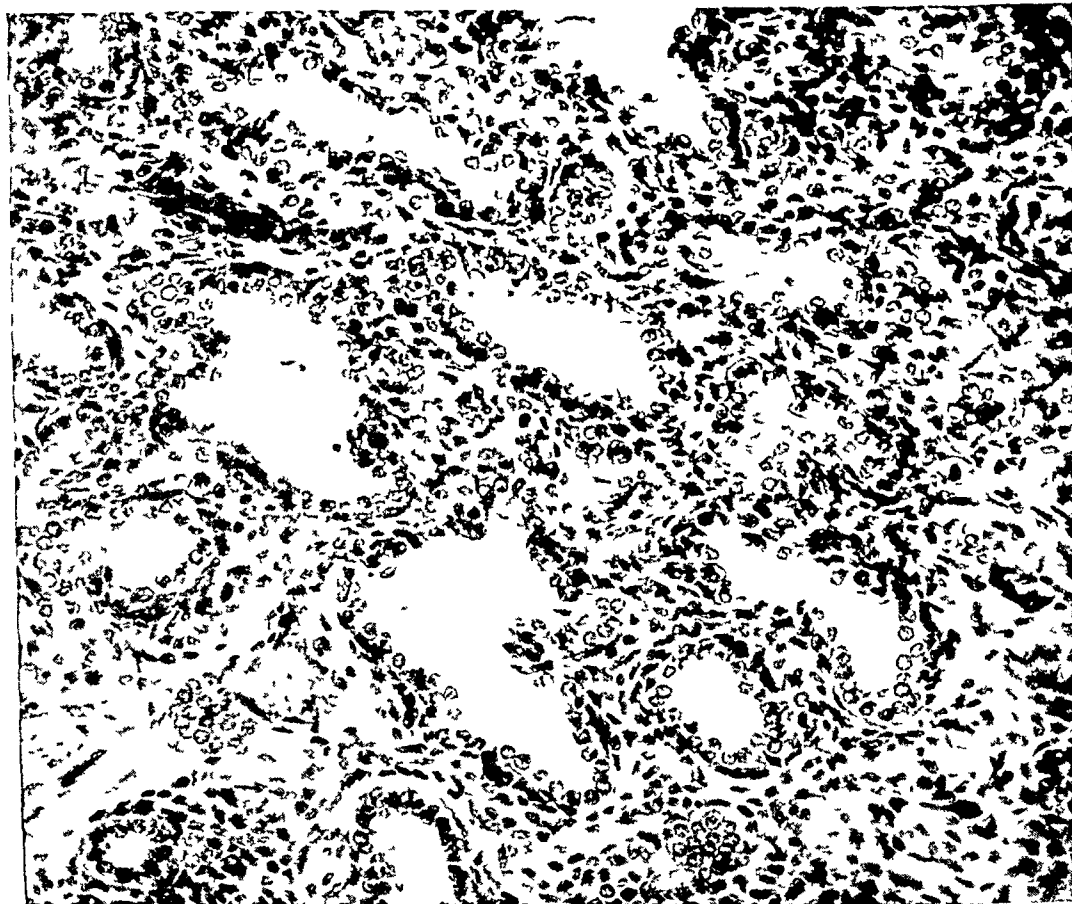


Fig 2—Lung of an infant who lived fifty minutes (weight, 550 Gm , length, 30 cm , intrauterine age, 22 weeks) of the previable premature group Cuboidal cells form the lining of all the alveoli



Fig 3—*A*, lung of a stillborn infant (weight, 1,225 Gm , length, 39 cm , intrauterine age, 21 weeks) of the viable premature group There is a transition from cuboidal cells lining the alveoli in some areas to flattened cells in other areas *B*, lung of an infant who lived 45 days (weight, 850 Gm , length, 38 cm , intrauterine age, 17 8 weeks) of the previable premature group There is a transition from cuboidal cells lining the alveoli in some areas to flattened cells in other areas



the grouping devised by Scammon, instead of relying solely on the computed gestational age. This classification, briefly is as follows:

- 1 Abortive Group
  - Length less than 28 cm
  - Weight less than 400 Gm
  - Gestation less than 22 weeks
- 2 Premature Group
  - (a) Previaible
    - Length from 28 to to 34.9 cm
    - Weight from 400 to 999 Gm
    - Gestation from 22 through 27 weeks
  - (b) Viable
    - Length from 35 to 46.9 cm
    - Weight from 1,000 to 2,499 Gm
    - Gestation from 28 through 36 weeks
- 3 Full Term Group
  - Length from 47 to 54 cm
  - Weight from 2,500 to 4,500 Gm
  - Gestation from 37 to 43 weeks
- 4 Postmature Group
  - Length more than 54 cm
  - Weight more than 4,500 Gm
  - Gestation more than 43 weeks

In any case in which the three variables did not fall into one group, the infant was considered as in that group in which two of the three factors were present.

The entire abortive group of 14 fetuses was characterized by a complete cuboidal lining of the alveoli. Of the 12 infants in the previaible premature group, half presented an immature cuboidal lining, while the remaining ones revealed a combination of cuboidal and flat cells. In the viable premature group 9 infants were noted to have a mixed lining, and the other 9 revealed a complete mature, flattened cell layer. In 4 of the 5 infants in the full term group a mature, flattened "epithelium" was observed, while in 1 infant there was a mixture of cuboidal and flat cells. The single postmature infant revealed a complete flattened lining, such as is seen in the adult lung.

*Type of Cells in Relation to Liveborn and Stillborn Infants*—Twenty of the infants studied were born alive. Thirty were stillborn.

An investigation of the cases of livebirth revealed that the alveolar lining cells were flat and mature in only 7 cases. Four of the infants who were born alive and lived up to three and one-half hours had a complete layer of immature, cuboidal cells lining the alveoli. Nine infants had a transitional type of cell lining, composed of both cuboidal and flat cells.

The clinical history of the 4 infants who were born alive and were later noted to have an immature alveolar cell lining incompatible with respiration revealed that the duration of life was from a few minutes to three and one-half hours. The weights varied from 265 to 980 Gm. Two of these infants belonged to the abortive group. Two belonged to the previable premature group. All 4 infants were delivered in spontaneous abortion, 2 in breech presentation. The postmortem examination showed prematurity and atelectasis in 3 of these infants and, in addition, subdural and subtentorial hemorrhage in 1.

Among the 9 infants presenting a mixed lining of both cuboidal and flattened cells were the infants who lived forty-five and fifteen days, respectively. Their deliveries were spontaneous and normal. The infant who lived forty-five days weighed 850 Gm. at birth and belonged to the previable premature group. At autopsy, severe icterus and serous hepatitis were present. The infant who lived fifteen days weighed 1,395 Gm. and belonged to the viable premature group. The postmortem examination revealed anomalies of the bladder, intersexuality and absence of the abdominal muscles.

The duration of life of the remaining infants of this group was from twenty minutes to six hours. The weights of these infants varied from 750 to 1,750 Gm. Two of the infants belonged to the previable premature group. Five belonged to the viable premature group. The deliveries of the entire group were spontaneous and normal. Two of the subjects were twins, and 1 was born in breech presentation, there was associated polyhydramnios, but the delivery was short and uncomplicated. There were 2 others who were in breech presentation in this group, and 1 who was in compound presentation. Since the alveolar "epithelium" composed of cuboidal and flat cells in this group had the potentiality of respiration, the possible causes of death were carefully investigated. In addition to the previously mentioned infants showing, at autopsy, anomalies and serous hepatitis, 4 revealed only atelectasis and prematurity, and, of the others, 1 infant presented a subtentorial hemorrhage and 1 a subcapsular hemorrhage of the liver.

The duration of life in the 7 infants whose alveolar lining was completely mature and capable of respiration was from one hour and twenty-five minutes to twenty-seven hours. The weights varied between 910 and 2,915 Gm. Four of these infants were premature but viable, 1 was previable and premature, 1 was full term, and 1 was postmature. Three of the infants were born by easy spontaneous deliveries. Two were born by cesarian section, one section was done in a case of hypertension and the other in a case of placenta praevia. One infant was in breech presentation at birth and was delivered with forceps on the after-coming head. The last case was one of placenta praevia in which

the infant was delivered by version and extraction. At autopsy 5 infants revealed only prematurity and atelectasis, 1, in addition, presented slight hypertrophy of the heart. The other 2 infants showed pathologic changes as follows: one, an achondroplastic dwarf, subdural hemorrhage and macrocephaly, the other, bilateral hydronephrosis, distention of the urinary bladder and congestion of the brain.

Of the 30 stillborn infants, 16 presented immature cuboidal "epithelium" incompatible with respiratory function, 6 had both cuboidal and flat cells, and 8 revealed a mature flattened lining.

Investigation of the 8 infants who were stillborn and had a mature alveolar lining capable of function revealed that 3 were full term and 5 were premature but viable. Six of these infants were delivered in a normal manner. One, in a case of placenta praevia, was delivered by cesarian section, and another was delivered by postmortem section on a mother who died of tuberculous meningitis. At autopsy the infant in the case of placenta praevia revealed subcapsular hemorrhages of the liver and lungs, indicating asphyxia. The mother of an infant in whom only atelectasis was present died six hours post partum with a diagnosis of sickle cell anemia. One infant died from cerebral hemorrhage. The other infants of this group revealed only atelectasis at death.

#### COMMENT

The study of the lungs of 50 newborn infants revealed that a complete cuboidal alveolar lining is incompatible with life. This type of lining was exclusively present in every infant in the abortive group and in 50 per cent of those in the previable premature group.

The persistence of life in the previable premature group—for example, in this investigation, 1 previable infant weighing only 850 Gm. lived forty-five days—may be accounted for by a partially mature alveolar lining. In the previable group, although half of the group presented a completely immature lining, the other half of the group revealed a mixture of cuboidal cells and mature flattened cells.

In the viable premature group, however, there was persistence of some immature, cuboidal alveolar cells, which might account for the death of an otherwise normal infant. Only half of the infants in the viable group revealed a completely mature alveolar lining, the others showed a mixed layer of cuboidal and flat cells. It was interesting that in this viable group there was not one infant that presented a complete immature, cuboidal lining, in other words, the potentiality for the function of respiration was always present.

In the full term group, in which viability is expected, 4 of the 5 infants revealed a fully matured cell lining, 1 infant, however, presented a mixed lining in which cuboidal cells still persisted.

This investigation also showed that the period of transition from cuboidal to flattened "epithelium" occurred in the greatest percentage of the cases between the twenty-fourth and thirty-third week of gestational age, or at the time that the previable infant becomes viable. Over 60 per cent of the infants between 24 and 28 weeks and 40 per cent between 28 and 33 weeks of intrauterine age presented this mixed type of alveolar lining.

Among the liveborn infants it was noted that the longest duration of life in those whose alveoli were composed entirely of immature, cuboidal cells was three and one-half hours. The duration of life in those infants whose lungs were lined by a mixture of cuboidal and flattened cells was forty-five days and fifteen days in 2 and up to fourteen hours in the others. Furthermore, of the 20 infants who were born alive, 12 gave no evidence of disease, anomaly or injury at autopsy. In these 12 cases of "unexplained death" the duration of life was from a few minutes to twenty-one hours. Two of the subjects were in the abortive, 2 in the previable premature and 8 in the viable premature group. In 3 of the 12 cases there was a complete immature cell lining, incompatible with life, in 4 a mixture of cuboidal and flattened cells and in 5, a mature alveolar lining which was capable of respiratory function. Therefore, 7 of the 12 "unexplained deaths" could be accounted for by either a completely immature or partially immature alveolar cell lining, the remaining 5 could not be explained.

In comparing the infants who were born alive with those who were stillborn, it was found that only 4 of the 20 liveborn infants as compared with half of the 30 stillborn ones had a complete immature cuboidal lining. It was also noted that 35 per cent of the liveborn infants as compared with 20 per cent of the stillborn ones presented a mature flat alveolar lining. In other words, a potential respiratory functioning, lining was noted more frequently in the infants who were born alive than in those who were stillborn.

#### SUMMARY

The entire abortive group and the greatest percentage of the previable premature group revealed an immature, cuboidal alveolar lining, which is not compatible with respiration. The greatest percentage of the full term group revealed a flattened, mature cell lining such as that seen in the adult lung.

The transition from cuboidal immature to flattened mature alveolar lining cells was noted most frequently between the twenty-fourth and thirty-third week of intrauterine life, or at the time that the previable premature infant becomes viable.

Cuboid, nonrespiratory functioning cells persisted in 50 per cent of the viable premature infants and in 20 per cent of the full term infants. This persistence of immature cells in viable infants as a pathologic phase of an earlier physiologic process is significant in the deaths of otherwise normal infants. In a study of 12 unexplained deaths in which no pathologic condition could be discerned at postmortem examination, it was observed that 7 of the infants had a complete or partial immature alveolar cell lining.

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# GOLGI APPARATUS OF THE THYROID GLAND

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A reticular material called the Golgi apparatus is present in all differentiated cells. It can be demonstrated under the microscope by special fixation of the tissue and by impregnation with silver. This reticular material is particularly well developed in the cells of the nervous system and in the secretory cells of glands. Study of the Golgi apparatus of the thyroid gland under various biologic conditions has yielded some definite information as to the relation between the size of the apparatus and the physiologic activity of the cell.

Hirschlerowa<sup>1</sup> observed hypertrophy of the Golgi apparatus in the thyroid cells of amphibian larvae undergoing metamorphosis. She later found an enlarged Golgi apparatus in the thyroid gland of a patient with exophthalmic goiter. Further studies by Ludford and Cramer<sup>2</sup> demonstrated that exposure to cold brought about hypertrophy of this reticular material in the thyroid glands of rats and mice. It seems to be generally agreed that enlargement of the Golgi apparatus in the cells of glands is evidence of increased secretory activity.

Other investigators have attempted to attach some significance to the position of the Golgi apparatus within the cell. Its usual position is between the nucleus and the secretory surface. Cowdry<sup>3</sup> suggested that in the case of the thyroid gland a basal position of the apparatus might be an indication of a secretion of toxic products directly into the blood stream. Other workers<sup>4</sup> could not ascribe any significance to the position of the apparatus and on the whole this line of investigation proved unfruitful. There is, however, no doubt of the value of an estimation of the size of the Golgi apparatus of the thyroid gland,

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From the Section on Surgical Pathology, the Mayo Clinic

1 Hirschlerowa, Z. *Ztsch f Zellforsch u mikr Anat* **6** 234, 1927

2 Cramer, W., and Ludford, R. I. *J Physiol* **61** 398, 1926. Ludford, R. J., and Cramer, W. *Proc Roy Soc London s B* **104** 28, 1928

3 Cowdry, E. V. *Am J Anat* **30** 25, 1922

4 (a) Okkels, H. *Acta path et microbiol Scandinav* **9** 1, 1932. (b) Gilman, J. *South African J M Sc* **1** 97, 1935. (c) Hirschlerowa<sup>1</sup>

as Okkels and Krogh and Lindberg<sup>5</sup> showed in their study of goiters. In a series of papers they called attention to the constancy of the finding of an enlarged Golgi apparatus in the cells of thyroid glands removed from patients with toxic goiters. Included were some thyroid glands from patients with exophthalmic goiter and some from patients with adenomatous goiter with hyperthyroidism. The size of the Golgi apparatus did not seem to be affected by the preoperative administration of iodine, although the glands usually showed regression to a state approaching that of the colloid thyroid in appearance.

The Golgi apparatus of thyroid gland cells has been studied in various types of goiter and the classification referred to in a previous article by one of us (Broders<sup>6</sup>) has been used to differentiate abnormal thyroid conditions. This classification which evolved from the clinical and pathologic conceptions of the thyroid gland of Wilson,<sup>7</sup> MacCarty,<sup>8</sup> H. S. Plummer,<sup>9</sup> Boothby,<sup>10</sup> and Broders<sup>6</sup> importantly differentiates adenomatous goiter from exophthalmic goiter.

#### MATERIAL AND METHOD

The Golgi apparatus was examined in 73 thyroid glands removed at operation. DaFano's<sup>11</sup> technic was used to demonstrate it. Portions of glands were taken from representative areas, part of which were prepared by the DaFano method. Fresh sections were stained by Terry's<sup>11a</sup> neutral polychrome methylene blue, and sections fixed in 4 per cent solution of formaldehyde by hematoxylin and eosin, the sections had been cut by the freezing method. Estimations of the size of the Golgi apparatus were made without previous knowledge of the clinical diagnoses. The diagnosis of cellular hypertrophy in these cases was made from frozen sections by members of the staff of the Section on Surgical Pathology. The size of the Golgi apparatus was designated either as "enlarged" or "not enlarged," no attempt being made to differentiate various degrees of enlargement. In doubtful cases the classification was "not enlarged." Table 1 summarizes the pathologic observations.

#### HISTOLOGIC AND CYTOLOGIC OBSERVATIONS

*Parenchymal Cellular Hypertrophy of the Thyroid Gland*—The gross and microscopic characteristics of the thyroid gland which are associated with various types of thyroid disease are well known. We have

5 Krogh, M., and Lindberg, A. L. *Acta path et microbiol Scandinav* **9** 21, 1932. Krogh, M., Lindberg, A. L., and Okkels, H. *ibid* **9** 37, 1932. Krogh, M., and Okkels, H. *Compt rend Soc de biol* **112** 1694, 1933. Okkels, H., and Krogh, M. *Acta path et microbiol Scandinav* **10** 118, 1933. Okkels, H. *Compt rend Soc de biol* **112** 1691, 1933, footnote 4a.

6 Broders, A. C. *Texas State J Med* **31** 608, 1936.

7 Wilson, L. B. *Am J M Sc* **136** 851, 1908, **147** 344, 1914, **165** 738, 1923.

8 MacCarty, W. C. *New York State J Med* **12** 595, 1912.

9 Plummer, H. S. *J A M A* **61** 650, 1913, *Am J M Sc* **146** 790, 1913.

10 Boothby, W. M. *Endocrinology* **5** 1, 1921, *J A M A* **74** 1600, 1920.

11 DaFano, C. *J Roy Micr Soc*, 1920, pt 2, p 157.

11a Terry, B. T. *J Lab & Clin Med* **14** 519, 1929.

based the histologic descriptions which are briefly presented in this paper on a previous summary.<sup>6</sup> The pathologic diagnosis of parenchymal cellular hypertrophy of the thyroid is regularly made in cases in which the gland is removed from a patient with exophthalmic goiter. The usual microscopic picture of parenchymal cellular hypertrophy of the thyroid is one of follicles lined with high cuboidal or columnar epithelium. There is papillary infolding of the epithelium, and colloid is less abundant. The administration of compound solution of iodine, however, alters the pathologic picture, and at the time of resection a much less severe process than originally was present is seen so far as the hypertrophic changes are concerned.

Now that iodine, as advocated by H. S. Plummer,<sup>12</sup> has come into almost universal use in the preoperative period, one sees less frequently

TABLE 1—*Microscopic Observations on the Golgi Apparatus in Seventy-Three Thyroid Glands*

Histologic Diagnosis	Total Cases	Golgi Apparatus	
		Enlarged	Not Enlarged
Parenchymal cellular hypertrophy of the thyroid	32	31	1
Single or multiple colloid and fetal adenomas (with or without intra adenomatous hypertrophy and also with various types of degeneration) in a thyroid with parenchymal hypertrophy	3	3	0
Single or multiple colloid and fetal adenomas (with various types of degeneration) in a colloid thyroid with intra adenomatous parenchymal hypertrophy	8	7	1
Single or multiple colloid and fetal adenomas (with various types of degeneration) in a colloid thyroid	29	17	12
Colloid thyroid	1		1

that marked cellular hypertrophy which was so well known to older pathologists. As Giordano<sup>13</sup> and Rienhoff<sup>14</sup> showed, iodine produces cytologic regression of the gland. The epithelium becomes flattened, colloid accumulates in the follicles, and the gland to a large extent assumes the appearance of a colloid goiter. There are varying degrees of this return of the gland to a more normal state under iodine therapy but in some instances after prolonged administration of iodine the microscopic picture simulates almost completely that of the colloid thyroid. Although great cellular hypertrophy cannot be seen, there are certain characteristics of this gland, such as lymphocytic infiltration of the interfollicular tissue, the presence of germ centers and the general arrangement of the acini, by which the pathologist may recognize that exoph-

12 Plummer, H. S. J. A. M. A. **80** 955, 1923

13 Giordano, A. S. Arch. Path. **1** 881, 1926

14 Rienhoff, W. F., Jr. Arch. Surg. **13** 391, 1926



thalmic goiter has been present. In exophthalmic goiter the gland usually does not contain adenomas, in less than a fourth of the cases, however, fetal adenomas or adenomatous tissue may be present in a thyroid gland exhibiting diffuse parenchymal hypertrophy. In some cases of adenomatous goiter, also, parenchymal hypertrophy may occur with or without intra-adenomatous hypertrophy. This condition is pathologically different from other types of adenomatous thyroid disease and clinically may be distinguished as exophthalmic goiter, as we shall point out later.



Fig. 1—Parenchymal cellular hypertrophy of the thyroid gland ( $\times 1,200$ )  
Note hypertrophy of the Golgi apparatus

For 35 glands the diagnosis of parenchymal cellular hypertrophy of the thyroid with or without regenerative hyperplasia was made (table 1). In 3 glands adenomatous tissue was present. The Golgi apparatus was found to be enlarged in 34 of these 35 glands. In some it was greatly enlarged, in others, less so. Figure 1 shows a portion of the thyroid gland removed from a patient with exophthalmic goiter. Three weeks before operation this patient's basal metabolic rate was plus 73 per cent.

Iodine was given in the usual doses during this three weeks' preoperative period. While the patient's general condition improved with the lowering of the basal metabolic rate, it will be seen in figure 1 that definite cellular hypertrophy is still present and that the Golgi apparatus is markedly enlarged. The one gland in this series on which the diagnosis of parenchymal cellular hypertrophy of the thyroid gland was made and in which no hypertrophy of the Golgi apparatus was seen will be considered later.

*Adenomatous Thyroid Gland*—Adenoma of the thyroid gland may be single, but usually it is multiple. Invariably, various types of degeneration may be seen in the adenoma. Some adenomas are encapsulated and anatomically demarcated from the surrounding colloid thyroid tissue in which they are situated. They contain fetal as well as colloid follicles. In cases in which adenomatous thyroid tissue produces clinical hyperthyroidism, hypertrophy of the cells lining the follicles in the adenomas is observed in a third of the cases. In such an event the process is spoken of as "intra-adenomatous hypertrophy," and columns of high cuboid cells are seen in the follicles, with infolding papillary projections of the epithelium, which are similar in appearance to the generalized process in parenchymal cellular hypertrophy of the thyroid gland. The colloid thyroid tissue surrounding the adenoma in this condition does not have hypertrophic epithelial changes. In approximately two thirds of the cases in which the diagnosis of adenomatous goiter with hyperthyroidism is made intra-adenomatous cellular hypertrophy is not present. In cases of adenomatous goiter, then, the presence or absence of clinical hyperthyroidism cannot always be correlated with the pathologic observations. To state the matter in other words. Many adenomatous thyroid glands producing clinical hyperthyroidism will not show evidence of cellular hypertrophy in the adenoma.

We have mentioned that diffuse parenchymal cellular hypertrophy involving extrinsic thyroid tissue may occur in a nodular goiter with or without intra-adenomatous hypertrophy. Such a condition is clinically and pathologically different from adenomatous thyroid with hyperthyroidism and essentially similar to the parenchymal cellular hypertrophy of the thyroid which does not contain adenomas.

Forty thyroid glands containing adenomatous tissue or adenomas were studied. In 3 of these diffuse hypertrophic changes were present in the extra-adenomatous tissue, and they have already been considered in the parenchymal cellular hypertrophy group. Eight of the 37 adenomatous thyroid glands under consideration showed intra-adenomatous hypertrophy (table 1). In 7 of these 8 glands the Golgi apparatus was found to be enlarged. The remaining 29 glands showed

no intra-adenomatous hypertrophy, although the Golgi apparatus was enlarged in 17

It at once became evident that a large number of adenomatous thyroid glands with enlargement of the Golgi apparatus do not exhibit cellular hypertrophy. As we will show, many of these glands were producing hyperthyroidism when they were removed. No enlargement of the Golgi apparatus in the colloid thyroid tissue extrinsic to the adenoma was found in the glands studied from this standpoint. The number of glands

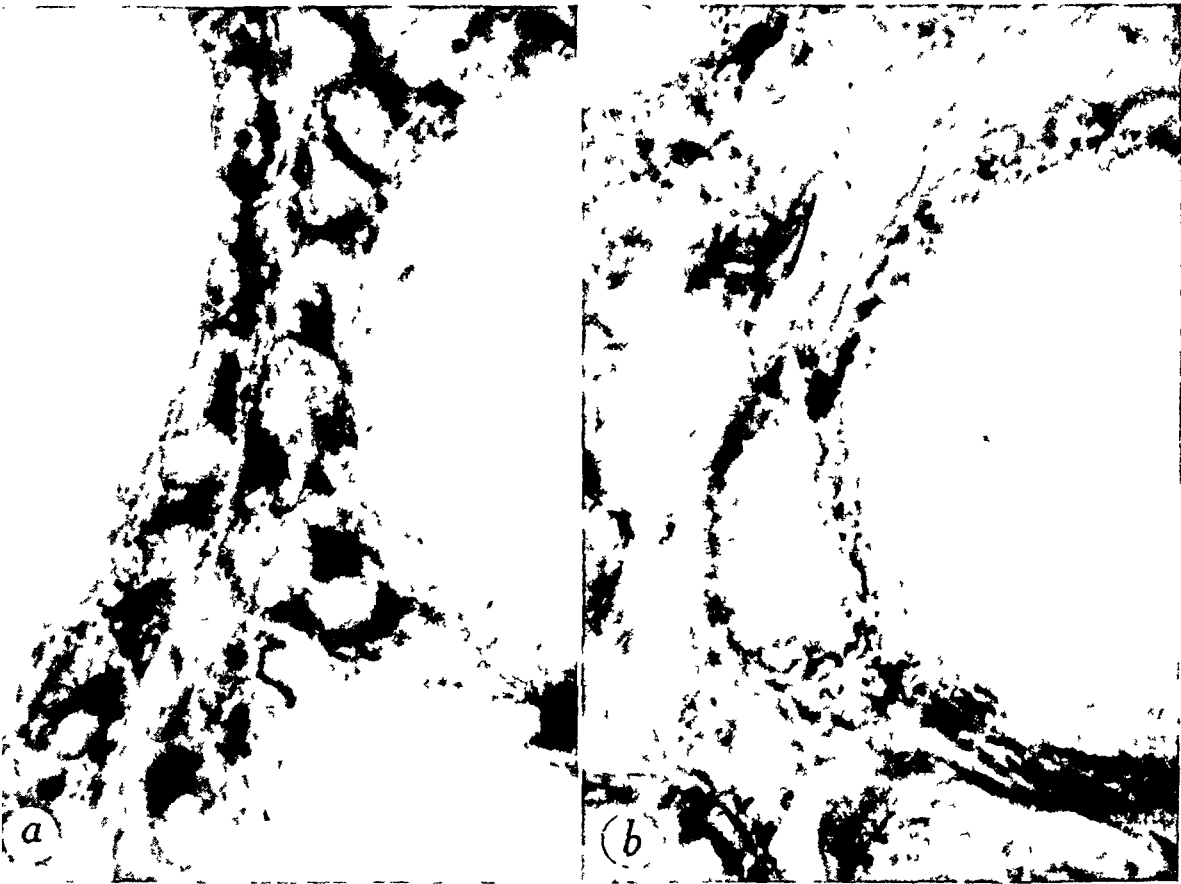


FIG 2—(a) Adenoma of the thyroid gland of a patient with hyperthyroidism, showing hypertrophy of the Golgi apparatus ( $\times 1,200$ ) (b) Thyroid tissue extrinsic to the adenoma shown in *a*, the Golgi apparatus is not hypertrophied here

studied in respect to this point of the size of the Golgi apparatus in the extra-adenomatous tissue we do not feel is sufficient to make the observations conclusive. This most interesting aspect of the study requires further work. Figure 2 *a* shows the Golgi apparatus in an adenoma of a thyroid gland removed from a patient with hyperthyroidism. The apparatus is dense and large and comparable to that seen in the gland with diffuse parenchymal hypertrophy. In certain instances

the enlarged Golgi apparatus in the adenoma appears to have a shape different from that seen in the thyroid presenting parenchymal cellular hypertrophy. This observation is now being subjected to further study. In figure 2b the Golgi apparatus in the extra-adenomatous tissue of the same gland is seen to be small and difficult to define with certainty.

*Colloid Thyroid Gland*—Only 1 specimen of colloid goiter was obtained, this condition being rarely encountered today.<sup>6</sup> Microscopic examination of a colloid goiter reveals a series of follicles lined with flattened or low cuboidal epithelium and containing abundant colloid. Okkels<sup>4a</sup> found the Golgi apparatus in this type of gland to consist of a fine strand, situated usually on the border of the nucleus which is toward the lumen. It was impossible to prepare suitable photomicrographs from our single specimen. In the very inactive cell of the colloid goiter, small strands of silver are seen with great difficulty. In many cells the Golgi apparatus unquestionably could not be seen, in fact, from our studies it seems that the apparatus may be observed easily and distinctly only when it is hypertrophied.

#### CORRELATION OF THE CLINICAL, HISTOLOGIC AND CYTOLOGIC OBSERVATIONS

While our series of cases is small, certain findings seem significant.

*Parenchymal Cellular Hypertrophy of the Thyroid Gland*—The 35 thyroid glands on which the pathologic diagnosis of parenchymal cellular hypertrophy was made were removed from patients who had exophthalmic goiter clinically (table 2). As we have stated, the administration of compound solution of iodine preceded operation in each case. Usually, iodine was given for a period of from seven to fourteen days before operation, in some cases, however, treatment was prolonged over several months. In many of the cases in which the gland was removed at operation the pathologic diagnosis of parenchymal cellular hypertrophy was qualified by the statement that little cellular hypertrophy was left. As we have indicated, the diagnosis of hypertrophy of the thyroid gland associated with the disease exophthalmic goiter does not entirely depend on the degree of cellular hypertrophy but may be made from the general morphologic appearance of the gland even though marked regressive changes have occurred under iodine medication. All these glands except one had enlargement of the Golgi apparatus in the cells lining the follicles. This gland was removed from a patient who had been receiving iodine for five months before operation. On microscopic examination there was little evidence of cellular hypertrophy left.

From our study of this group of thyroid glands with parenchymal cellular hypertrophy it appears that cellular regression is accompanied

by a decrease in the size of the Golgi apparatus. In many of the follicles of the glands removed from patients with exophthalmic goiter who had been treated with iodine the cells showed marked regression and are flattened. In these cells no enlarged Golgi apparatus can be seen. This seems logical and explains our findings in the single case of exophthalmic goiter in which no enlarged Golgi apparatus was found in the cells of the gland removed at operation. In the majority of cases, however, areas are found with cells showing enlargement of the Golgi apparatus. We cannot agree with Okkels' statement that the size of the Golgi apparatus is not altered by iodine medication even though cellular regression is marked. We do note, however, that the enlargement of the apparatus in a thyroid gland of the hypertrophic parenchymal type is a more prominent feature than the cellular hypertrophy in the glands from certain patients who have obtained marked benefit from iodine therapy. It must

TABLE 2—*Clinical Diagnoses and Microscopic Observations in Seventy-Three Cases of Thyroid Disease*

Clinical Diagnosis	Cases	Cases in Which Cellular Hypertrophy Was		Cases in Which Golgi Apparatus Was	
		Present	Absent	Enlarged	Not Enlarged
Exophthalmic goiter	35	5		21	1
Adenomatous goiter with hyperthyroidism	21	7	14	19	2
Adenomatous goiter without hyperthyroidism	16	1	15	5	11
Colloid goiter	1		1		1

be remembered, however, that administering iodine seldom is sufficient to restore normal metabolic conditions, and cellular hyperfunction is present in practically all of the glands removed at operation.

*Adenomatous Thyroid Glands*—Twenty-one of the 37 adenomatous thyroid glands in this study were removed from patients with hyperthyroidism as determined by the usual methods (table 2). Of these 21 glands, only 7 showed intra-adenomatous cellular hypertrophy. On the other hand, the Golgi apparatus was found to be enlarged in 19, and in all these the enlargement was definite. These observations suggest that the size of the Golgi apparatus is a more delicate method of estimating cellular function than the usual histologic method. In 2 cases no enlargement of the Golgi apparatus was seen in the sections obtained for study.

Sixteen of the adenomatous thyroid glands were obtained at operation in cases in which the clinical diagnosis of adenomatous goiter without hyperthyroidism had been made (table 2). Figure 3 shows a portion

of an adenoma removed from a patient without clinical hyperthyroidism. The Golgi apparatus is enlarged and gives evidence of increased cellular function in this adenoma. The finding of enlargement of the Golgi apparatus in 5 of 16 of these cases of adenomatous goiter without hyperthyroidism (table 2) is of especial interest, and although the group is small, it suggests that hyperfunction of certain portions of the adenomatous thyroid may occur without producing a sufficient degree of hyperthyroidism to permit its recognition with certainty.



Fig. 3—Adenoma of the thyroid gland of a patient without clinical hyperthyroidism, showing hypertrophy of the Golgi apparatus ( $\times 1,200$ )

#### COMMENT

From the foregoing study of 73 cases of goiter, it seems true that the Golgi apparatus of the thyroid cells are of different sizes under varying conditions of activity and in various types of thyroid disease. In the cells of the normal gland and in the cells of the colloid thyroid Okkels<sup>4a</sup> found the Golgi apparatus to be a small filament. Our observation in flattened cells which histologically appear only normally functioning or less so is that the Golgi apparatus cannot be clearly seen. The contrast between such cells, however, and those of definitely hyper-

functioning tissue, even under low power magnification, is so striking that one can immediately differentiate the two. When the Golgi apparatus is enlarged, it is easily seen (figs 4 *a* and *b*, 5 *a* and *b* and 6), and the enlarged Golgi apparatus in hyperfunctioning tissue has a striking appearance.

We have found the Golgi apparatus enlarged in practically all the glands showing parenchymal hypertrophy removed from patients with exophthalmic goiter (34 of 35). Okkels<sup>1a</sup> stated that he invariably found

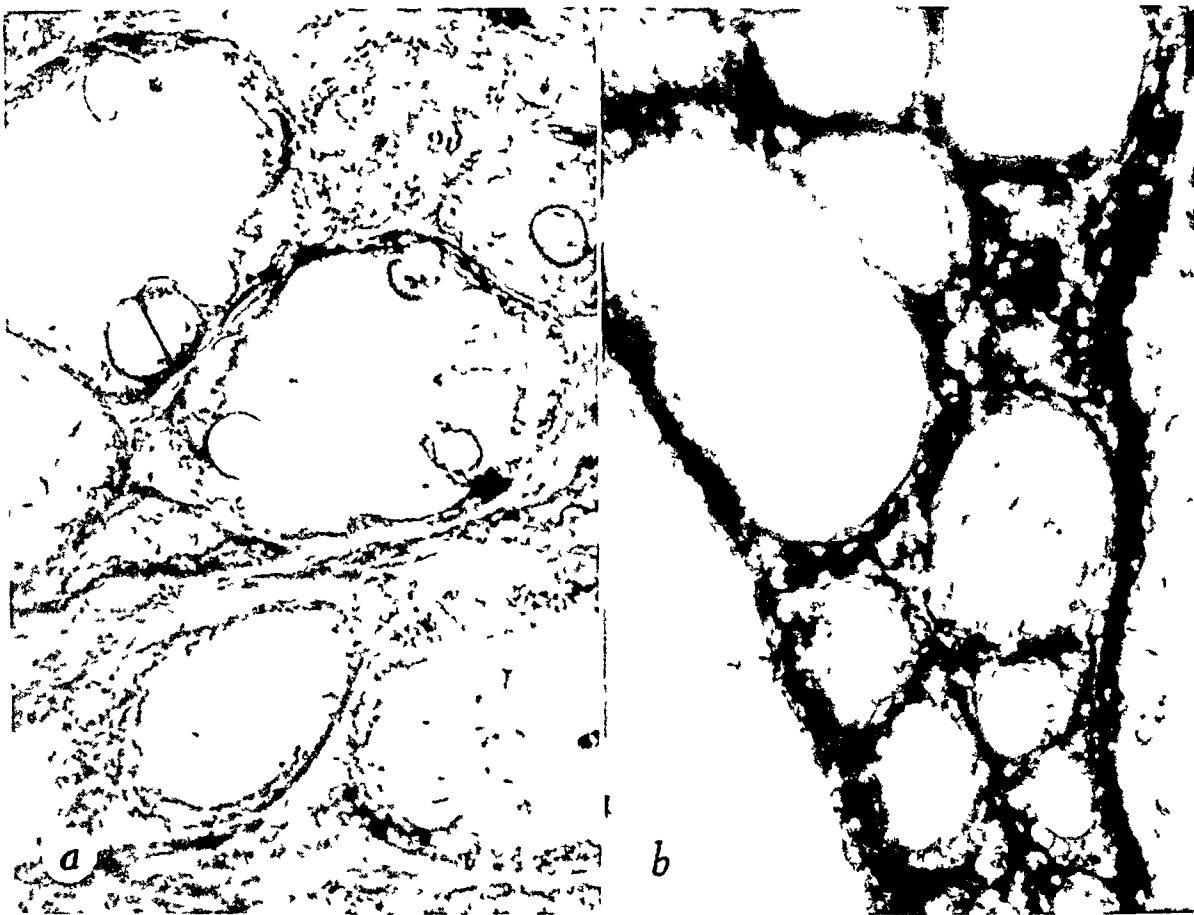


Fig 4—(a) Colloid thyroid tissue with no hypertrophy of the Golgi apparatus ( $\times 485$ ). (b) Colloid thyroid tissue extrinsic to a hyperfunctioning adenoma, with no hypertrophy of the Golgi apparatus ( $\times 485$ ).

enlargement of the apparatus of the thyroid cell in the presence of exophthalmic goiter, even after almost complete return of the gland to a state cytologically resembling colloid thyroid in the patient who had received iodine for some time. As a result, he was of the opinion that there is constant cellular hyperfunction and that iodine does not affect cellular function but rather effects an accumulation of colloid in the follicle by some other mechanism. This was also the observation of

Gilman,<sup>4b</sup> who recommended abandoning the use of iodine on the basis of these cytologic observations

It is indeed hard to subscribe to these conclusions in the light of certain considerations. Obviously, in the majority of patients who have been operated on but who are still suffering from hyperthyroidism the basal metabolic rate is elevated. The cells are therefore still hyperfunctioning.

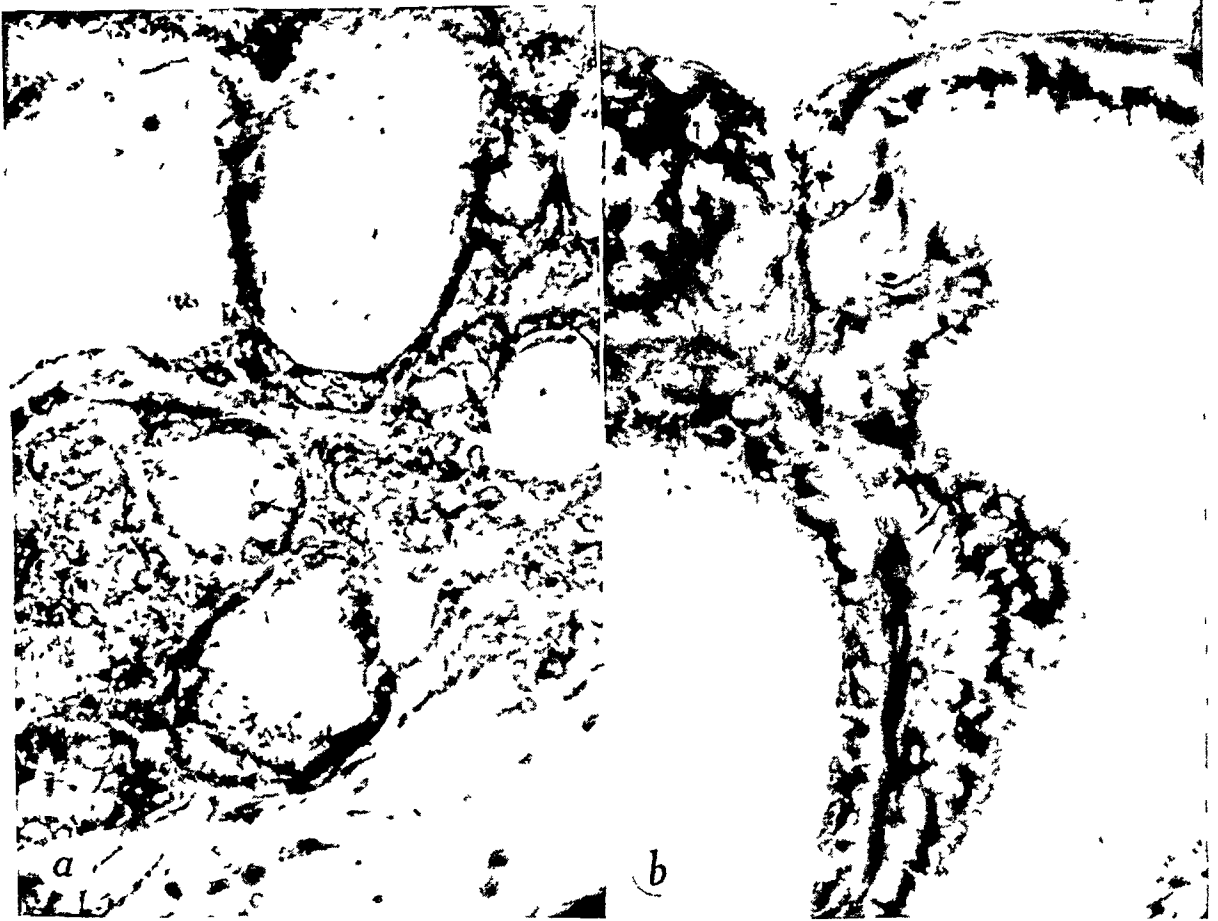


Fig 5—(a) Adenoma of a thyroid without cellular hypertrophy removed from a patient without hyperthyroidism, the Golgi apparatus is not hypertrophied ( $\times 485$ ). (b) Parenchymal cellular hypertrophy of the thyroid, the Golgi apparatus is hypertrophied ( $\times 485$ ).

The Golgi apparatus is enlarged and appears as a dense black mass in the cells of the adenomatous tissue which is hyperfunctioning and producing clinical hyperthyroidism. In 19 of 21 glands evidence of hyperfunction in the adenoma was obtained by the finding of a hypertrophied Golgi apparatus. Sufficient cellular hypertrophy to be considered evidence of hyperfunction could be found in only 7 of these glands. While the number of cases studied does not allow us to say what the exact correlation is between a recognizable degree of hyperthyroidism



and enlargement of the Golgi apparatus in adenoma of the thyroid, the correspondence seems fairly high, and enlargement of the apparatus is more definite evidence of hyperthyroidism than can be obtained from an estimation of cell size by the usual histologic methods routinely employed in the pathologic laboratory

The finding of enlargement of the Golgi apparatus in the adenomatous tissue of thyroid glands not producing hyperthyroidism is of special interest. We occasionally find evidence of cellular hypertrophy in adeno-



Fig 6—Adenoma of a thyroid removed from a patient with hyperthyroidism, the Golgi apparatus is hypertrophied ( $\times 485$ )

mas removed at operation from patients without clinical hyperthyroidism. In the case of nodular goiter the clinical diagnosis of hyperthyroidism is sometimes difficult to make, and it may in the final analysis depend on whether the basal metabolic rate is elevated above some arbitrary figure, such as plus 15 per cent. Boothby<sup>15</sup> expressed the opinion that there are, however, a considerable number of patients whose symptoms can-

15 Boothby, W. M. Unpublished data

not be definitely classified either as typical or as severe enough to warrant the diagnosis of hyperthyroidism. The basal metabolic rate may be from five to fifteen points above the patient's own mean, which, of course, may be definitely below the standard mean, which is the average of many persons. These persons may be suffering from mild degrees of hyperthyroidism, which may be extremely difficult to recognize with certainty. Hertzler<sup>16</sup> expressed the opinion, based on clinical observation, that a large number of goiters are toxic without giving evidence of it from the basal metabolic rate. It has been the observation of Haines and W. A. Plummer<sup>17</sup> for a long time that many patients with adenomatous goiter without hyperthyroidism feel immeasurably improved after thyroidectomy.

There is, then, considerable indication clinically that mild hyperthyroidism is present in a large group of persons with adenomatous goiter in spite of the fact that the condition frequently cannot be classified as hyperthyroidism. Boothby<sup>15</sup> recorded some evidence of this mild activity of adenomatous thyroid tissue. From the standpoint of the pathologic anatomy of the adenomatous thyroid gland, it is a reasonable conception that the nondegenerative portions of the adenoma are functioning beyond the capacity of normal cells or of the cells in thyroid tissue extrinsic to the adenoma. Boothby suggested a reasonable explanation for the physiologic activity of the adenomatous gland which will allow a presumption of increased production of thyroxin by the adenoma in the patient with adenomatous goiter without resulting in hyperthyroidism. He has postulated that in the event of increased production of thyroxin by the adenoma a mechanism which regulates the normal total production of thyroxin per day (0.4 mg.) decreases the activity of the extrinsic or normal tissue in the gland. This process of compensatory functional retardation of the extrinsic glandular tissue may be exhausted by an ever increasing size of the abnormally functioning adenomas. In such an event clinical hyperthyroidism may result. With this concept of the diseased adenomatous goiter in mind, it becomes clear that the development of hyperthyroidism may be slow. A concomitant degeneration of certain portions of the adenoma is another factor in delaying the onset of increased production of thyroxin. These postulates with regard to adenomatous goiter present a picture of a process progressive toward the hyperthyroid state.

#### SUMMARY

A study of the Golgi apparatus of the thyroid gland has been made in 73 thyroid glands from patients who came to operation for goiter. Hypertrophy of the Golgi apparatus was found in 34 of 35 glands.

16 Hertzler, A. E. *Surgical Pathology of the Thyroid Gland*, Philadelphia, J. B. Lippincott Company, 1936.

17 Haines, S. F., and Plummer, W. A. Personal communication to the authors.

removed from patients with exophthalmic goiter and in 19 of 21 glands removed from patients with adenomatous goiter with hyperthyroidism. Hypertrophy of the apparatus was also found in 5 of the 16 adenomatous goiters removed from patients who were not judged to have hyperthyroidism. Appraising the size of the Golgi apparatus in the thyroid gland would therefore seem to offer a more delicate method of estimating cellular and glandular function than ordinary methods.

This study has purposely not been made on selected pathologic groups. Certain general information has been obtained which opens avenues for considerable study of the various types of goiter individually, using this method of demonstrating the Golgi apparatus. Unfortunately, the method of preparing and staining sections to demonstrate the Golgi apparatus is too time-consuming to be used in the routine pathologic diagnosis of surgical material.

# CHEMOTACTIC PROPERTIES OF TUBERCULO- PHOSPHATIDE AND TUBERCULO- POLYSACCHARIDE

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It is well established that in vitro the tubercle bacillus exerts a positive chemotropic action on both human and animal polymorphonuclear neutrophils and that in experimental infections of animals there is early exudation of these cells into the infected regions. In a previous paper it was reported that tuberculo-protein when adsorbed on kaolin or charcoal strongly attracts human polymorphonuclear leukocytes.<sup>1</sup> The present study is concerned with the phosphatide and polysaccharide fractions of the tubercle bacillus.

## METHODS

Tuberculo-phosphatide was obtained from the H-37 strain of human tubercle bacilli.<sup>2</sup> The polysaccharide<sup>3</sup> was prepared from strain A-10 and has properties similar to those of the crude polysaccharide described by Heidelberger and Menzel.<sup>4</sup> Both preparations belong in the group of water-alcohol-ether extracts of living tubercle bacilli which has been designated residue I.<sup>5</sup>

Both rabbit and human leukocytes were studied. Rabbit polymorphonuclear leukocytes were secured by injecting sterile physiologic solution of sodium chloride into the peritoneal cavity and were suspended in homologous plasma. Human polymorphonuclear leukocytes were obtained from finger blood of presumably healthy adults.

The method employed to study the chemotropism of the leukocytes has been described in detail elsewhere.<sup>1</sup> A small amount of the substance to be tested was placed on a clean glass slide. A drop of plasma-leukocyte suspension or finger blood was placed on a cover slip and spread over the test substance, which thus

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1 Wartman, W B. Arch Path **26** 694, 1938

2 Crowder, J A, Stodola, F H, Pangborn, M C, and Anderson, R J. J Am Chem Soc **58** 636, 1936

3 Anderson, R J, Reeves, R E, and Stodola, F H. J Biol Chem **121** 649, 1937

4 Heidelberger, M, and Menzel, A E. O. J Biol Chem **118** 79, 1937

5 Sabin, F R, and Joyner, A L. J Exper Med **68** 853, 1938

formed a small target on the slide. Because the phosphatide and polysaccharide are soluble in blood plasma it was necessary to use as a target some chemotactically inert substance saturated with the test solution. The substances employed were kaolin and titanium dioxide. Control experiments were made on the same slide, with pure washed kaolin or titanium dioxide being used as targets.

The fraction to be tested was dissolved in fresh triple-distilled water and allowed to come into contact with kaolin or titanium dioxide, which was then used as the target. Each sample of kaolin or titanium was tested to determine whether or not phosphatide or polysaccharide was actually present, and only those preparations were used which contained the desired fraction. The movements of leukocytes near the target were observed under the microscope in a warm chamber at 37 C., and the course of each cell was recorded with the aid of a drawing ocular at one minute intervals for a period of ten minutes. The index of chemotropism was defined as the ratio between the net approach of a cell either toward or away from the target to the actual distance traveled by the cell. In each group of experiments the results were expressed as the mean chemotactic index of all the cells observed.

TABLE 1—*Chemotactic Properties of Phosphatide from the Tubercle Bacillus*

Substance Tested	Experiment		Control	
	Cells	Mean Chemotactic Index with Standard Error	Cells	Mean Chemotactic Index with Standard Error
Pure phosphatide	20 human leukocytes	$-0.09 \pm 0.06$	39 human leukocytes	$-0.03 \pm 0.07$
Pure phosphatide	38 rabbit leukocytes	$+0.15 \pm 0.04$	122 rabbit leukocytes	$-0.05 \pm 0.01$
Phosphatide on titanium dioxide	111 rabbit leukocytes	$+0.43 \pm 0.03$	137 rabbit leukocytes	$+0.02 \pm 0.06$
Phosphatide on kaolin	110 rabbit leukocytes	$0.00 \pm 0.01$	88 rabbit leukocytes	$-0.08 \pm 0.04$

## RESULTS

Three different experiments were carried out with the phosphatide. In the first the undissolved powdered phosphatide was used as the target. In the second and third experiments titanium dioxide and kaolin moistened with dilute solutions of phosphatide were employed as targets.

When powdered tuberculophosphatide was used as the test material, most of the leukocytes in the vicinity of the target showed a decrease in motility and soon became immobile (table 1). Shortly many of the cells died. The cells which showed motion moved slowly, and the chemotactic index was not significantly different from the value obtained for leukocytes observed in fields remote from the target.

When tuberculophosphatide and titanium dioxide were used, the leukocytes were weakly attracted to the target. The chemotactic index was  $+0.43 \pm 0.03$ . Control experiments with titanium dioxide gave a chemotactic index of approximately zero. Similar experiments in which kaolin was used as an opaque substance gave a chemotactic index of

$0.00 \pm 0.01$ , indicating random motion. The kaolin control experiments likewise showed random motion.

The results of the experiments with the tuberculopolysaccharide in which kaolin was used as an inert substance indicate that this fraction weakly repelled the leukocytes (table 2). Again, the kaolin control showed random motion.

#### COMMENT

These experiments indicate that concentrated, undiluted tuberculo-phosphatide is toxic for both human and rabbit leukocytes, since it reduces the motility of the cells and finally kills them. When, however, the phosphatide is diluted, weak attraction is observed. The polysaccharide, on the other hand, elicits weak negative chemotaxis.

It thus seems probable that in vitro the protein fraction of the tubercle bacillus is chiefly responsible for attraction of polymorpho-

TABLE 2—*Chemotactic Properties of Polysaccharide from the Tubercle Bacillus*

Substance Tested	Experiment		Control	
	Cells	Mean Chemo- tactic Index with Standard Error	Cells	Mean Chemo- tactic Index with Standard Error
Polysaccharide on kaolin	132 human leukocytes	$-0.34 \pm 0.05$	120 human leukocytes	$+0.05 \pm 0.04$
Polysaccharide on kaolin	71 rabbit leukocytes	$-0.34 \pm 0.06$	37 rabbit leukocytes	$+0.02 \pm 0.10$

nuclear neutrophils.<sup>1</sup> As far as could be determined this fraction is not harmful to the cells. The polysaccharide, on the other hand, does not strongly attract neutrophils and may even weakly repel them. The action of the phosphatide, however, is complicated by its markedly injurious effect. Probably polymorphonuclear leukocytes migrate toward the phosphatide but are killed before reaching it.

Dixon, McCutcheon and Czarnetsky<sup>6</sup> obtained similar results with fractions prepared from a strain of hemolytic streptococci. They showed that the fraction which produces antibodies in experimental animals (protein-carbohydrate complex) also causes positive chemotaxis of rabbit leukocytes, while a carbohydrate fraction and a "protein-free, nonantigenic, crystalline stable hemolysin" elicit no chemotaxis. Under different experimental conditions Sabin<sup>7</sup> reported that all the fractions of the tubercle bacillus cause an outpouring of leukocytes when they are injected into guinea pigs (subcutaneous, intracutaneous

<sup>6</sup> Dixon, H. M., McCutcheon, M., and Czarnetsky, E. J. *Am. J. Path.* **13**: 645, 1937.

<sup>7</sup> Sabin, F. R. *Physiol. Rev.* **12**: 141, 1932.

and intraperitoneal injections) Sabin noted that the medium in which the fractions were suspended also caused emigration of leukocytes into the peritoneal cavity, but to a lesser degree. This discrepancy in results is at present unexplained but may be due to the difference in experimental conditions.

#### SUMMARY

The chemotactic properties of a phosphatide and a polysaccharide fraction of the tubercle bacillus have been studied *in vitro*. Under the conditions of the experiments it was found that the tuberculophosphatide in concentrated form is toxic for both human and rabbit neutrophils, but in suitable dilution it causes weak attraction of these cells. The tuberculopolysaccharide causes weak negative chemotaxis of the leukocytes.

# HISTOLOGIC CHANGES IN THE RENAL ARTERIOLES OF HYPERTENSIVE RABBITS

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In a recent review Goldblatt<sup>1</sup> stated that in renal hypertension, "if there is no accompanying renal insufficiency, the kidneys show no significant gross or microscopic changes detectable by usual methods" He added that the changes in the afibrillar cells of the preglomerular arterioles reported by Goormaghtigh and Grimson<sup>2</sup> are interesting as the only changes occurring in hypertension, but stated that their significance awaits elucidation Goormaghtigh<sup>3</sup> more recently reported that the juxtaglomerular apparatus of normal rabbits contains chiefly afibrillar cells which exhibit a normal glandular cycle of vacuolation and acidic and basic granulation Moreover, he reported that in 3 rabbits rendered hypertensive by the Drury technic there was an increase in number and size of these granular cells, an increase of afibrillar cells and formation of granular cells in the preglomerular arterioles Goormaghtigh suggested that the afibrillar cells are responsible for arteriolar tone and that the granular cells are the source of the pressor substance in hypertension In this report we wish to offer corroboration and extension of Goormaghtigh's findings

## MATERIAL AND METHODS

This report is based on a study of kidneys from 39 rabbits, on which blood pressures were determined by the abdominal cuff method of McGregor<sup>4</sup> Of this group, 14 rabbits were "normal" or untreated, 12 received injections of aspartic acid, either intramuscularly or intravenously, 7 had one renal artery partially ligated by the Drury<sup>5</sup> technic, and 6 had one or both renal arteries partially ligated by a modification of Goldblatt's<sup>1</sup> method Eight of the "normal" or control rabbits

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1 Goldblatt, H Am J Clin Path **10** 40, 1940

2 Goormaghtigh, N, and Grimson, K S Proc Soc Exper Biol & Med **42** 227, 1939

3 Goormaghtigh, N Proc Soc Exper Biol & Med **43** 688, 1939

4 McGregor, L Arch Path **5** 630, 1928

5 Drury, O R J Exper Med **68** 695, 1938



had blood pressures ranging from 80 to 120 mm, and 6 exhibited mild spontaneous elevations of between 20 and 30 mm above a maximum normal pressure of 120 mm. In the group which received injections of aspartic acid, 7 rabbits had normal pressures and 5 showed elevations of from 20 to 30 mm. The remaining rabbits, on which partial ligation of the renal artery was done by either the Drury or the Goldblatt procedure, exhibited elevated pressures, ranging from 150 to 240 mm.

The kidneys were removed immediately after the rabbits were killed and fixed in Zenker's solution prepared with formaldehyde, sectioned 4 to 5 microns in thickness and stained according to Goldner's<sup>6</sup> modification of Masson's trichrome stain.

### RESULTS

In the 15 rabbits which had normal blood pressures throughout the period of observation (8 normal and 7 treated) the juxtaglomerular apparatus consisted of a few afibrillar or paucifibrillar cells and varying numbers of sparsely granular cells (*A*, fig 1). The granulations in the latter cells gave a basophilic reaction in some and an acidophilic reaction in others. The acidophilic granular cells ("A") were usually although not invariably, present in greater numbers than the basophilic granular cells ("B"). From one to three densely granular "A" cells were seen in the juxtaglomerular apparatus of some glomeruli and none in others. The "B" cells were invariably sparsely granular. Most of the so-called afibrillar cells of Goormaghtigh were found to contain faint basophilic stippling, however, these cells appeared much lighter than the typical "B" cells. Situated near the nucleus of each granular cell was a clear vacuole-like area containing one or more spherical or irregularly shaped, darkly staining structures (*A*, fig 1). This complex which will henceforth be referred to as the vacuolar complex, resembled a similar structure found in the basophilic cell of the pituitary gland. In the "B" cells and sparsely granular "A" cells this complex was small and often indefinite, however, in the densely granular "A" cells it was larger and quite distinct. The afibrillar and paucifibrillar cells were devoid of this complex. In these normal kidneys, as well as in those of the experimental rabbits to be described subsequently, the granular and afibrillar cells were located almost exclusively in the outer cortex.

A striking change was seen in the juxtaglomerular apparatus and renal arterioles of rabbits with blood pressures of 170 mm or over (*M*<sub>8</sub>, *M*<sub>15</sub>, *H*<sub>1</sub>, *H*<sub>2</sub>, *H*<sub>3</sub>, 163, 150). In these animals, particularly *M*<sub>8</sub>, *M*<sub>15</sub>, and *H*<sub>2</sub>, there was a marked increase in size and number of "A" cells in the juxtaglomerular apparatus (*B* and *C*, fig 1), apparently at the expense of the afibrillar and "B" cells, for the latter were reduced in number as compared with the normal. The density of granulation in many of the "A" cells was greatly augmented, giving them a brilliant red granular appearance, which made them readily visible under magnifications of 100 diameters. Some of the "A" and "B" cells in these

<sup>6</sup> Goldner, J. *Am J Path* 14:237, 1938.



Figure 1

Photomicrographs,  $\times 1,635$  The glomerulus in all photographs of the juxtaglomerular apparatus lies in the field above that shown

*A*, juxtaglomerular apparatus of control rabbit 7 (blood pressure, 110 mm) On the right are acidophilic granular cells, most of which are densely granular A vacuolar complex is indicated by the arrow On the left are several sparsely granular "B" cells In the lower cell of this group some of the granules are acidophilic

*B*, juxtaglomerular apparatus of experimental rabbit M15 (partial ligation of both renal arteries, Goldblatt type, blood pressure, 240 mm) All cells of this juxtaglomerular apparatus are of the acidophilic granular type The three or four cells at the upper left are sparsely granular Note the vacuolation of the large granular subendothelial cell on the left

*C*, juxtaglomerular apparatus of experimental rabbit M8 (partial ligation of one renal artery, Goldblatt type, blood pressure, 175 mm) The large clear binucleate cell with faint granulations at the lower left is a "B" type, but a few of the granules stain acidophilic The granular cell to the left and above this one is also binucleate, but the nuclei do not show in this plane Except for the light cells in the lower center, all other cells are of the "A" type

*D* cross section through an arteriole of rabbit M15 (see note on *B* for protocol) Note the large granular subendothelial "A" cell



Figure 2

Photomicrographs,  $\times 1,635$

*E*, longitudinal section through a branching arteriole of rabbit M 15 (see note on *B* for protocol) All the granular cells present are acidophilic in type Note the gradation in density of granulation

*F*, section through an arteriole of experimental rabbit H3 (partial ligation of one renal artery, Drury type, blood pressure, from 200 to 163 mm) Note the large vesicular nucleus of the medial cell at the left of center and the variability in size and staining reaction of other medial cells Typical-staining smooth muscle cells are seen at the extreme lower left

*G*, section through a branching arteriole of rabbit M15 (see note on *B* for protocol) Note the large number of densely granular "A" cells

*H*, acidophilic granular cells from glomerular arteriole of rabbit M15 (see note on *B* for protocol) The arrow indicates an enlarged vacuolar complex

*I*, section through arteriole of rabbit H2 (Drury type, blood pressure, 186) Note the mitotic figure in the afibrillar cell

animals were binucleate (*C*, fig 1) The vacuolar complex in the "A" cells was enlarged and prominent (*H*, fig 2), and vacuolation of the cytoplasm was more extensive (*B*, fig 1) The "B" cells were not enlarged and differed from the normal only in some instances in which there was an apparent development of acidophilia in the granules, with occasional distinctly acidophilic granules The preglomerular arterioles were markedly altered by the presence of densely granular and developmental stages of the "A" cells in the media, especially at the points of branching (*G*, fig 2 and *D*, 1) In this location a complete transitional series from clear afibrillar to mature "A" cells was present (*E*, fig 2) Granular cells were never present in the arterioles of rabbits with normal pressures Scattered singly in the media were also cells with faint basophilic stippling Furthermore, the number of typical

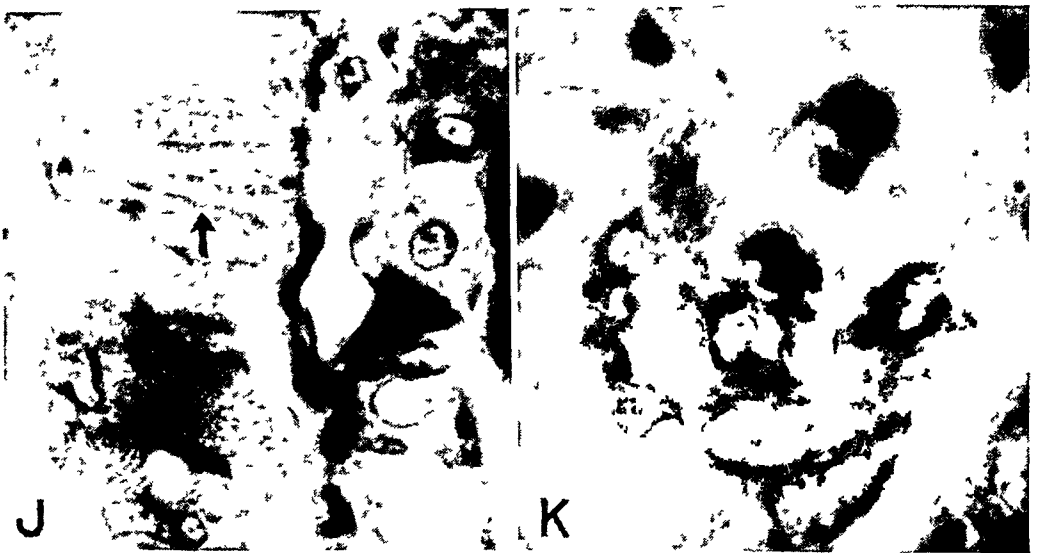


Figure 3

Photomicrographs,  $\times 1,635$

*J*, longitudinal section through an arteriole of rabbit M8 (see note on *C* for protocol) The arteriole on the left is cut tangentially, and the lumen lies beneath the cells Note the densely granular cells at the lower left, one contains a large vacuole The arrow indicates a clear cell containing many fuchsinophilic bodies On the right is the left wall of an adjacent arteriole Most of these cells are granular or vacuolated

*K*, juxtaglomerular apparatus of rabbit 163 (partial ligation of renal artery Goldblatt type, blood pressure, 156 mm) Note the muddy cytoplasm and the absence of distinct granulation in the juxtaglomerular cells

staining smooth muscle cells was reduced, these apparently being replaced by afibrillar cells or cells of the granular series (*J*, fig 3) In animal M<sub>8</sub>, and to a lesser extent in the others, some of the clear cells contained spherical fuchsinophilic bodies, which are interpreted as indicating secretory exhaustion of granular cells of the "A" type (*J*, fig 3)

Mitosis of afibrillar cells in the media was encountered only in animal H<sub>1</sub> (I, fig 2), which, although high blood pressure developed in it, did not have even the normal number of granular cells in the juxtaglomerular apparatus. However, the number of afibrillar and paucifibrillar cells was greatly increased in this animal. In animals H<sub>2</sub>, 150 and 163, in which blood pressures were elevated for periods ranging from four to twelve months, there were widespread tubular necrosis and fibrosis of glomeruli. The juxtaglomerular apparatus of the affected glomeruli in these animals was reduced in size and contained only darkly staining cells which were neither typical granular cells nor typical smooth muscle cells (K, fig 3). "A" cells were present in the preglomerular arterioles and were increased in number in the juxtaglomerular apparatus of the few relatively normal glomeruli. In rabbit H<sub>3</sub>, the juxtaglomerular apparatus contained increased numbers of "A" cells, and these cells were also present in the glomerular arterioles, however, the "A" cells contained very few acidophilic granules. The small number of densely or moderately granular cells and the large number of sparsely granular cells, in view of the blood pressure records of this animal, suggest a reversion of "A" cells to the afibrillar or paucifibrillar type. These afibrillar cells were abundant in the media of the arterioles (F, fig 2). The blood pressure records of H<sub>1</sub> reveal an initial pressure of 200 mm following removal of the normal kidney and a subsequent gradual drop over a period of three and one half months to 162 mm.

The changes in the juxtaglomerular apparatus and preglomerular arterioles were not so pronounced in animals with only moderate hypertension (140 to 160 mm) following partial ligation of the renal artery. In these animals there was a moderate increase in number and size of "A" cells of the juxtaglomerular apparatus, but the granulation of these cells was not markedly increased. The size of the granules was, however, distinctly larger in many of these cells. The number of afibrillar and "B" cells seemed to be decreased, and "A" cells were rarely found in the arterioles. However, there was a distinct increase of afibrillar cells in the preglomerular arterioles, which is, perhaps, the most noticeable change in the kidneys of these animals.

The rabbits which exhibited mild spontaneous elevations of pressure whether of the "normal" group or of the group receiving injections of aspartic acid, revealed only a very slight increase of "A" cells in the juxtaglomerular apparatus and a moderately increased number of afibrillar cells in the arterioles. The cause of these mild spontaneous elevations is the subject of another communication.<sup>7</sup>

#### COMMENT

Our observations on kidneys of normal and hypertensive rabbits suggest that the granular "A" cells in the juxtaglomerular apparatus

<sup>7</sup> Dunihue, F W Anat Rec (supp) **73** 64, 1939

and also in glomerular arterioles of hypertensive animals are secretory cells. The variation in the density of granulation of the "A" cells in normal animals and the increase in size as well as density of granulation in hypertensive rabbits support this conclusion. Further evidence is provided by the behavior of the vacuolar complex, which is usually enlarged and prominent in the densely granular "A" cells, especially in hypertensive rabbits. And finally the presence of a variable number of fuchsinophilic bodies in some of the clear cells of the periglomerular arterioles of acutely hypertensive rabbits is interpreted as indicating secretory exhaustion of granular cells. That the latter cells are probably formed from densely granular "A" cells is borne out by the finding of occasional fuchsinophilic bodies and enlarged acidophilic granules in some of the "A" cells. Therefore we concur with Goormaghtigh in holding these cells to be secretory.

If we accept the "A" cells as being secretory, their function as judged by location in the juxtaglomerular apparatus and periglomerular arterioles seems to be concerned with glomerular blood flow. The increase in size, number and secretory activity of the "A" cells in these locations in hypertensive rabbits further suggests such a function. Of course, it cannot be stated with certainty whether the activity of the "A" cells is cause or effect with respect to the elevated blood pressure. However, it seems reasonable on the basis of histologic evidence to assume, as does Goormaghtigh, that these cells produce a pressor principle. In this connection it is suggestive that the renal pressor substance (renin) is found only in extracts of the kidney cortex.<sup>8</sup> Under normal conditions the production of pressor substance, as judged by the appearance of the "A" cells, is probably not great. But in hypertensive animals these cells are increased in number and activity, forming glandular tissue, the total mass of which must be considerable. The products of secretion of such a mass of cells might conceivably affect the systemic blood pressure.

In both the normal and hypertensive kidney there is considerable evidence to indicate that the afibrillar, the "B" and the "A" cells form a developmental series. That there is a relationship between the "A" and "B" cells seems unquestionable. They both possess a vacuolar complex, and "B" cells have been observed which contain a few acidophilic granules. Although the nature of this relationship is at present not entirely clear, it seems that the "B" cells may form "A" cells. The afibrillar cells, although they do not possess a vacuolar complex certainly give rise to "A" cells in the arterioles of hypertensive rabbits as evidenced by the closely graded series from afibrillar to densely granular "A" cells. The evidence for the formation of "B" cells from afibrillar cells is not so clearcut, but this seems likely in view of the

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<sup>8</sup> Pickering, G. W., and Prinzmetal, M. *Clin. Sc.* **3**: 211, 1938.

gradations in granularity found in the afibrillar and "B" cells. We suggest that the afibrillar cells are the probable stem cells of the granular series. This is indicated by the increase of afibrillar cells in the arterioles of moderately hypertensive rabbits and the presence in the same location in acutely hypertensive animals of mature and developing granular cells. The fact that mitoses were observed only in afibrillar cells also argues for this interpretation. In view of this, we do not feel that afibrillar cells are directly concerned with maintaining local arterial tone as suggested by Goomaghtigh.

We have found no evidence to indicate the origin of the afibrillar cells in the juxtaglomerular apparatus, but when present in the arterioles these cells seem to have developed in locations ordinarily occupied by smooth muscle cells. This and the fact that the smooth muscle cells exhibit varying degrees of fibrillation suggest origin of afibrillar cells from smooth muscle. It is interesting to note in this connection that E. R. Clark and E. L. Clark<sup>9</sup> report the formation of smooth muscle cells from fibroblasts and the reversion of muscle cells to fibroblasts in their observations on capillary growth in transparent windows in rabbits' ears.

#### SUMMARY

The juxtaglomerular apparatus of the normal rabbit kidney contains afibrillar, basophilic granular and acidophilic granular cells which exhibit signs of cyclic secretory activity. The granular cells are present only in the juxtaglomerular apparatus of the normal kidney, but the afibrillar cells are present also, in small numbers, in the glomerular arterioles.

In hypertension, produced either by the Goldblatt or by the Drury technic, there is an increase in size and number of the acidophilic granular cells in the juxtaglomerular apparatus, and all three types of cells, especially the acidophilic granular, are present in the glomerular arterioles.

It is suggested that these three types of cells form a developmental series having a probable origin from smooth muscle cells, and that the acidophilic granular cells are the source of the renal pressor substance.

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<sup>9</sup> Clark, E. R., and Clark, E. L. *Am J Anat* **66** 1, 1940.

# EFFECTS OF THYROID AND CALCIUM THERAPY ON THE GROWTH OF SARCOMA TRANSPLANTS IN THYROPARATHYROIDECTOMIZED RATS

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Much literature has accumulated in the last few years with reference to the effect of thyroid and parathyroid hormones on the susceptibility of animals to growth of cancer and of tumor transplants. Murohara<sup>1</sup> found that removal of the thyroid gland had a tendency to inhibit the growth of a transplantable rabbit sarcoma. Paik<sup>2</sup> found that in parathyroidectomized rats the rate of growth of the Flexner-Jobling rat carcinoma was decreased. Matsuoka<sup>3</sup> reported the opposite observations on a similar tumor.

It is difficult to evaluate the seemingly confirmatory and contradictory results of different authors relative to the influence of various biologic substances on the growth of tumors because of the tremendous variability in the types of tumors used and in the characteristics of the biologic preparations. Even though the tissues of two tumors may be histologically similar, their reaction to chemotherapy may be markedly different. Another factor is the hereditary background of the host. Many authors have failed to state whether or not litter mates were used as controls in their experiments.

The conflict in results which have been reported relative to tumor growth in relation to metabolism makes it highly desirable that more detailed studies be made.

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1 Murohara, N. *Tr Jap Path Soc* **20** 658, 1930

2 Paik, T. S. *Am J Cancer* **15** 2756, 1931

3 Matsuoka, S. *Tr Jap Path Soc* **20** 657, 1930



The animals used in this study were from our own colony of Wistar Institute albino rats, which is maintained on a diet of fox chow, <sup>a</sup> furnished ad libitum, with bread and meat twice a week. At the age of 21 days they were weaned, and between the ages of 26 and 35 days their thyroid and parathyroid glands were removed. Thyroparathyroidectomy was carried out on the anesthetized animal. The neck was shaved and iodine applied to the depilated area. A midline incision was made, exposing the muscles on the anterior side of the neck, which were separated by blunt dissection exposing the thyroid. The lower pole of the left lobe of the thyroid was then lifted with a small pair of hand forceps, and tension was applied while it was being dissected free from the underlying tissues. Precautions were taken not to injure the recurrent laryngeal nerve during the dissection. The lifting and blunt dissection were continued until the isthmus was freed from its tracheal attachments. The right lobe was dissected in the same manner as the left. The skin was closed with interrupted sutures and iodine applied to the wound.

Immediately after the animals had been operated on, litter mates of the same sex and as near the same weight as practical were divided into four groups (table 1). Groups 1 and 2 (18 males and 18 females, respectively) received a diet of fox chow only. Groups 3 and 4 (20 males and 18 females, respectively) received a diet consisting of 99.98 per cent fox chow and 0.02 per cent desiccated thyroid. The animals were kept on their respective diets throughout the course of the experiment. During the first one hundred and fifty days of the experiment a few deaths occurred in each experimental group. On the one hundred and fiftieth day the 59 remaining animals were found to be distributed as follows (table 1): groups 1 and 2, 13 males and 16 females, respectively, and groups 3 and 4, 18 males and 12 females, respectively. At this time the animals were anesthetized with ether, and two small pieces of viable spindle cell sarcoma, each about the size of a grain of wheat, were placed beneath the skin in each animal through a dorsal incision near the level of the first lumbar vertebra. The sarcoma was a highly malignant growth of spindle and amorphous cells, obtained from the uterus of a rat and kept alive by transplanting small pieces beneath the skin through successive generations.

The quantity of thyroid used in the replacement therapy was that previously established <sup>4</sup> as adequate to produce a significant beneficial effect on the host. Indices to this beneficial effect were observed in weekly weights and in the femoral measurements obtained after the death of the animals. The weekly weights showed a stimulating effect on body growth at about the third week, which reached a maximum approximately seven weeks later. Beyond this time the thyroid-fed

### 3a The formula of the fox chow is as follows

Organic	Percentage	Inorganic	Percentage
Proteins	23	Iron	0.018
Fat	5	Magnesium	0.09
Fiber	4	Potassium	0.56
Ash	7	Silica	0.23
Nitrogen-free extract	54	Sodium	0.67
Moisture	7	Chlorine	0.68
		Phosphate	1.17
		Calcium	2.22
		Iodine	trace

<sup>4</sup> Patras, M. C. The Influence of Thyroid Feeding on the Skull Pattern of Thyroparathyroidectomized Rats, Thesis, University of Illinois Graduate School, 1939.

animals maintained their acquired stature. The second index, dealing with the size of the femur, revealed a significant increase in the length of this bone in those animals which had received thyroid therapy.

The weights obtained on the day the tumors were transplanted revealed an average difference of 78 and 43 Gm between the males and females, respectively, in favor of the groups receiving desiccated thyroid. The period of survival following the tumor inoculation varied from twenty-four to sixty-two days, with no significant difference between the sexes, nor was the longevity markedly influenced by the thyroid therapy.

The weekly weights obtained throughout the period of survival and at the time of death revealed a sharp increase beginning about two weeks after the tumor transplants were made. This rapid increase reached a maximum three to four weeks later, after which there was a slight decline.

As soon after death as practical, the tumors were removed and weighed. From these data was derived not only the average tumor weight but also the relation of the weight of the tumor to that of the host.

The average weight of tumor tissue per rat in the groups which did not receive thyroid therapy was found to be 69 Gm for both males and females. The groups receiving desiccated thyroid yielded significantly more tumor tissue per rat than the groups which did not receive thyroid. The average tumor weight per rat on thyroid was found to be 94 Gm for the males and 108 Gm for the females. This difference of 25 to 39 Gm in favor of an increase in tumor growth with thyroid therapy may be considered as corroborative of the view that thyroid stimulates new growth and susceptibility to cancer. Sugiura and Benedict<sup>5</sup> observed the rate of tumor growth to be very slow in animals fed a goitrogenic diet. Kosugi<sup>6</sup> claimed that thyroid deficiency exerted a marked inhibitory effect on the growth of the Kato rabbit sarcoma if this deficiency was established at the time of or before the tumor was transplanted. In general this work was confirmed by Nishida,<sup>7</sup> who added that substances which promote thyroid function seemed to accelerate tumor growth. Rohdenburg, Bullock and Johnston<sup>8</sup> obtained some evidence that thyroid deficiency decreased the susceptibility of animals to cancer. Bischoff and Maxwell,<sup>9</sup> on the other hand, were not able to find evidence that thyroparathyroidectomy influenced the rate of growth in a rat carcinoma.

A more detailed study of the data, taking into consideration the influence of thyroid on the host as well as on the tumor, does not support a conclusion that thyroid may have a specific effect on tumor growth. The average loss of body weight incurred by the animals between the time of the transplantation of tumor and their death was not found to be

5 Sugiura, K., and Benedict, S. R. *Am J Cancer* **23** 541, 1935.

6 Kosugi, K. *Tr Jap Path Soc* **27** 651, 1937.

7 Nishida, S. *Jap J Obst & Gynec* **18** 195, 1935.

8 Rohdenburg, G. L., Bullock, F. D., and Johnston, F. J. *Arch Int Med* **7** 491, 1932.

9 Bischoff, F., and Maxwell, J. *J Pharmacol & Exper Therap* **46** 51, 1932.

significantly different. The weights of the females on fox chow at the time of death (table 1) after the tumors had been removed revealed a loss of 25 per cent, while those receiving thyroid lost 27 per cent. The same general relation was observed in the male groups.

Since the thyroid therapy superimposed on a fox chow diet did not reveal a significant specific effect on the growth of tumor transplants the question of mineral balance, which is known to be disturbed,<sup>10</sup> was

TABLE 1—*Tumor Growth in Thyroparathyroidectomized Rats Fed Desiccated Thyroid*

Groups	Animals Operated On	Animals Operated on and Bearing Tumors	Average Weight of Animal at Beginning of Experiment	Average Weight of Animal on 150th Day	Average Weight of Animal and Tumor	Average Weight of Tumor	Average Weight of Animal Less Tumor
1 Males on fox chow	18	13	62	157	189	69	120
3 Males on fox chow and thyroid	20	18	62.5	235	290	94	196
2 Females on fox chow	18	16	57	132	167	69	98
4 Females on fox chow and thyroid	18	12	56	175	235	108	127

TABLE 2—*Tumor Growth in Thyroparathyroidectomized Rats Fed Desiccated Thyroid and Calcium Carbonate*

Groups	Animals Operated On	Animals Operated on and Bearing Tumors	Average Weight of Animal at Beginning of Experiment	Average Weight of Animal on 150th Day	Average Weight of Animal and Tumor	Average Weight of Tumor	Average Weight of Animal Less Tumor
5 Males on fox chow and calcium	22	20	60.7	165	220	68	152
7 Males on fox chow, calcium and thyroid	18	15	61	280	348	89	259
6 Females on fox chow and calcium	15	15	55.2	159	211	67	144
8 Females on fox chow, calcium and thyroid	17	14	57.5	203	284	71	213

considered. Calcium therapy (table 2) was therefore instituted in a group of 72 thyroparathyroidectomized rats. Until the time of operation these animals were treated in the same manner as those previously described. After the operation they were divided into four groups (table 2). Groups 5 and 6 (22 males and 15 females, respectively) received a diet consisting of 99 per cent fox chow and 1 per cent calcium carbonate. Groups 7 and 8 (18 males and 17 females, respectively)

<sup>10</sup> Tweedy, W. R., Templeton, R. D., Patras, M. C., McJunkin, F. A., and McNamara, R. W. *J. Biol. Chem.* **128**: 407, 1939.

received a diet comparable to that of groups 5 and 6 except for the addition of 0.02 per cent desiccated thyroid, which was substituted for an equivalent quantity of fox chow. Tumor transplants were made on the surviving animals one hundred and fifty days after the start of the experiment. Thirty-four days after these inoculations all animals were killed and the tumor tissue removed. This time of putting them to death was selected because the results obtained on groups without calcium therapy indicated that a maximum growth of host plus tumor was attained about thirty to thirty-five days after the transplantation of tumor.

A difference of 44 and 115 Gm for the females and males, respectively, was observed in favor of thyroid feeding at the time the tumor transplants were made. Approximately this same difference, with a rapid rise in the weight, which began between the second and third week after the inoculations, continued until the close of the experiment. The animals were killed for the tumor tissue before the occurrence of a marked decline in the weight of the hosts resulted from the tumor growth.

A comparison of the quantities of tumor tissue obtained from the various groups revealed a slight stimulating effect of thyroid. The average weight of tumor tissue taken from the animals which did not receive thyroid was 68 and 67 Gm, respectively, for males and females, while the groups receiving thyroid therapy yielded 89 and 71 Gm of tumor tissue, respectively, from males and females.

The stimulating effect of thyroid on tumor tissue loses its significance when its effect on the host is taken into consideration, since tumor tissue responds to thyroid therapy in practically the same manner as normal tissue.

#### SUMMARY

The influence of thyroid on tumor growth was proportional to its effect on the general metabolism, and the addition of 1 per cent calcium carbonate to the diet had no significant effect on the growth of the host or of the sarcoma transplant.

## Case Reports

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### CONGENITAL ATRESIA OF THE PANCREATIC DUCT WITH CYSTIC FIBROSIS OF THE PANCREAS

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BAITIMORF

Anderson<sup>1</sup> recently reviewed 49 cases of congenital pancreatic steatorrhea. 27 of these cases were already recorded in the literature and 22 were reported for the first time. The pathologic alteration of the pancreas in this series was fairly uniform, consisting usually of a lesion best described as cystic fibrosis. Occasionally complete fatty atrophy was found. Yet Anderson noted that the pancreatic ducts had been dissected infrequently and that an obstruction of the pancreatic ducts was observed in only a few cases. In 4 of her cases it was impossible to dissect the duct, but in only one of her protocols (no. 20) was definite atresia described, in 2 cases recorded in the literature stenosis of the duct was found near the orifice, and Anderson concluded that in 3 other instances definite atresia was observed. This last figure should be reduced to 1. She listed the reports by Tiling<sup>2</sup> and Benoit<sup>3</sup> as reports of 2 separate cases, yet they are descriptions of the same case. In the case reported by Siwe,<sup>4</sup> which Anderson lists as one of atresia of Wirsung's duct, there was a normal patent duct extending from the head to the tail of the pancreas, and Siwe considered the pancreatic lesion as due to "agenesis" of the excretory acinar tissue.

In all, therefore, there are only 4 cases recorded in the literature in which an obstructed pancreatic duct was seen to cause cystic fibrosis of the pancreas in infancy. A brief summary of the data on these cases follows.

Kornblith and Otani<sup>5</sup> reported the condition in a 5 day old infant. With serial microscopic sections they saw the main pancreatic duct, which was dilated throughout the body of the pancreas, suddenly narrow to a slitlike lumen in the head of the pancreas. Though the duct was stenotic, its lumen was not obliterated. Parmelee<sup>6</sup> noted in a 4½ year old girl that the duct at the head of the pancreas was "very narrow." Tiling<sup>2</sup> and Benoit<sup>3</sup> separately reported the condition in the same 7 month old infant. Actual atresia of Wirsung's duct was demonstrated in serial sections. The orifice of the duct was located in the duodenum beneath the common bile duct, the duct was then traced in the duodenal

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1 Anderson, D. H. *Am J Dis Child* **56** 344, 1938.

2 Tiling, W. *Arch f Kinderh* **106** 9, 1935.

3 Benoit, W. *Endokrinologie* **16** 313, 1935.

4 Siwe, S. S. *Deutsches Arch f klin Med* **173** 339, 1932.

5 Kornblith, B. A., and Otani, S. *Am J Path* **5** 249, 1929.

6 Parmelee, A. H. *Am J Dis Child* **50** 1418, 1935.

wall and was seen to branch, but it immediately narrowed down to nothing and ended without ever penetrating through the muscularis of the duodenum or entering the pancreas. Finally Anderson<sup>1</sup> in the protocol of case 20, that of a 3 year old girl, noted that a small pancreatic duct was found which on gross examination extended from the ampulla for 8 mm and was then "lost in fibrous tissue." No microscopic sections of this lesion were described.

Since the publication of Anderson's review, 2 other cases of pancreatic steatorrhea have appeared in the literature. In the case reported by Thomas and Schlutz<sup>7</sup> the pancreatic duct was dilated. The cause of the lesion was not found. In the case observed by Davie,<sup>8</sup> although Santorini's duct was patent Whistung's duct was thought to be occluded by fibrous tissue. Unfortunately, the pancreas was separated from the duodenum, and the orifice of the duct was not seen, nor was any histologic description of the duct recorded.

To make this summary of cases complete, I should like to add 5 cases of congenital cystic pancreatic fibrosis found by a review of the autopsy records of this hospital, these cases were noted briefly by Rich and Duft.<sup>9</sup> The precise obstruction was not demonstrated in any of these cases, but in 2 of them a suggestive microscopic lesion of the duct was seen, which had not been recognized grossly. In an autopsy on a 6½ month old boy the section of the ampulla of Vater showed a "multilocular system of glands suggesting a sort of papillomatous tumor growth" which might well have been the cause of the pancreatic lesion, although without serial sections this could not be established. Likewise, in an autopsy on a 3½ month old girl a section near the ampulla showed a large thick-walled pancreatic duct in which the mucosa was thrown up in papillomatous folds that narrowed the lumen, but in the absence of serial sections the nature of this lesion could not be determined.

From this survey it can be seen that a histologic picture of the lesion in the pancreatic duct causing cystic fibrosis of the organ is rare indeed. A case is reported, therefore, as in it serial sections were made of all the pertinent material. It seems advisable to add this case to the small number already described.

#### REPORT OF A CASE

A 10 month old white girl had always presented a feeding problem. She failed to gain well and vomited rather frequently. At 6 months she was admitted to the Harriet Lane Home with an infection of the upper respiratory tract and severe diarrhea. She was malnourished and dehydrated and had acute pharyngitis and otitis media. The diarrhea subsided in about two weeks. She ate well in the hospital, without vomiting, but did not gain well in spite of a large caloric intake. The stools were always large and dry, and an analysis of them showed poor absorption of fats. The dextrose tolerance was normal, but there was a tendency to alimentary glycosuria when the carbohydrate intake was large. There was a period of transient pyelitis, which was complicated by another attack of diarrhea. The baby had shown a tendency to wheeze, but no allergic reaction could be demonstrated. She was gaining fairly well when, nine days before her death, she

7 Thomas, J, and Schlutz, F W. *Am J Dis Child* **56** 336, 1938.

8 Davie, T B. *J Path & Bact* **46** 473, 1938.

9 Rich, A R, and Duft, G L. *Bull Johns Hopkins Hosp* **58** 212, 1936.

again acquired an infection of the upper respiratory tract, followed by bronchitis, bronchiolitis and pneumonia. The clinical impression was pancreatic deficiency, vitamin A deficiency secondary to poor absorption of fats, diffuse bronchitis, bronchiolitis and pneumonia.

*Pathologic Observations*—Autopsy was performed one and one-half hours after death. Only the important changes are noted here. The body weighed only 5.5 Kg. Nutrition was poor. There were no teeth. The abdomen was distended. The lungs showed foci of lobular consolidation, and pus-filled bronchioles were conspicuous. The liver was large and contained a good deal of fat. The gallbladder and bile ducts and the other viscera were normal. The lines of ossification of the bones were all straight and narrow.

The pancreas was the center of interest. It was left connected to the duodenum. It seemed to be of normal size and shape. No orifice corresponding to that of Santorini's duct was found in the duodenum. A minute orifice, thought to belong to Wirsung's duct, was seen adjacent to the ampulla of Vater. This was so narrow that a probe could not be passed. For this reason the pancreas was sectioned, but its ducts could not be seen, nor were any dilated channels or cysts noted at this time. In fact, the lobulation seemed about normal. However, a few hours after fixing the specimen in Zenker's fluid a striking change was seen. Instead of showing normal lobulation, the surface was covered with white foci varying in size from minute dots to spots with a diameter of 2.5 mm. The largest foci were easily seen to be cavities filled with inspissated material. When any of these cysts was probed it was found to end blindly, and there was no area that could be identified as a duct.

*Microscopic Observations*—Only the abnormalities are described.

(a) *Respiratory Tract* The mucosa of the trachea showed in places a thick layer of squamous epithelium replacing its normal columnar cells. Squamous epithelium even lined many of the ducts and acini of the mucous glands and was found in the main bronchi and also in tiny bronchioles in the depth of the lungs. The bronchioles were filled with an exudate of polymorphonuclear leukocytes. Their walls were thickened by infiltrating leukocytes and wandering cells. A few patches of peribronchial alveoli in both lungs were filled with purulent exudate.

(b) *Rib* The line of ossification of the rib was entirely normal.

(c) *Genitourinary Tract* The renal parenchyma was normal. The pelvic mucosa was greatly thickened, but the cells maintained their transitional character. The ureteral mucosa was also thick, but it too was normal transitional epithelium. The epithelium of the bladder formed a thicker layer than normal but showed no metaplasia. Thick squamous epithelium lined the vagina and cervix but did not extend into the uterus.

(d) *Gastrointestinal Tract* The mucosa of the ducts of the salivary glands was appreciably thickened, and in some places areas of squamous metaplasia were seen. No ducts were occluded, but some dilated acini were seen. The epithelium of the esophagus formed a much thicker layer than normal. Patches of mononuclear cells were seen in its submucosa. In frozen sections of the liver stained with scarlet red fine globules of fat were seen in every cell.

(e) *Pancreas and Duodenum* Serial sections showed the minute orifice identified grossly as the orifice of Wirsung's duct. The conclusion that this did not extend into the pancreas was substantiated, as microscopically (fig. 1) it

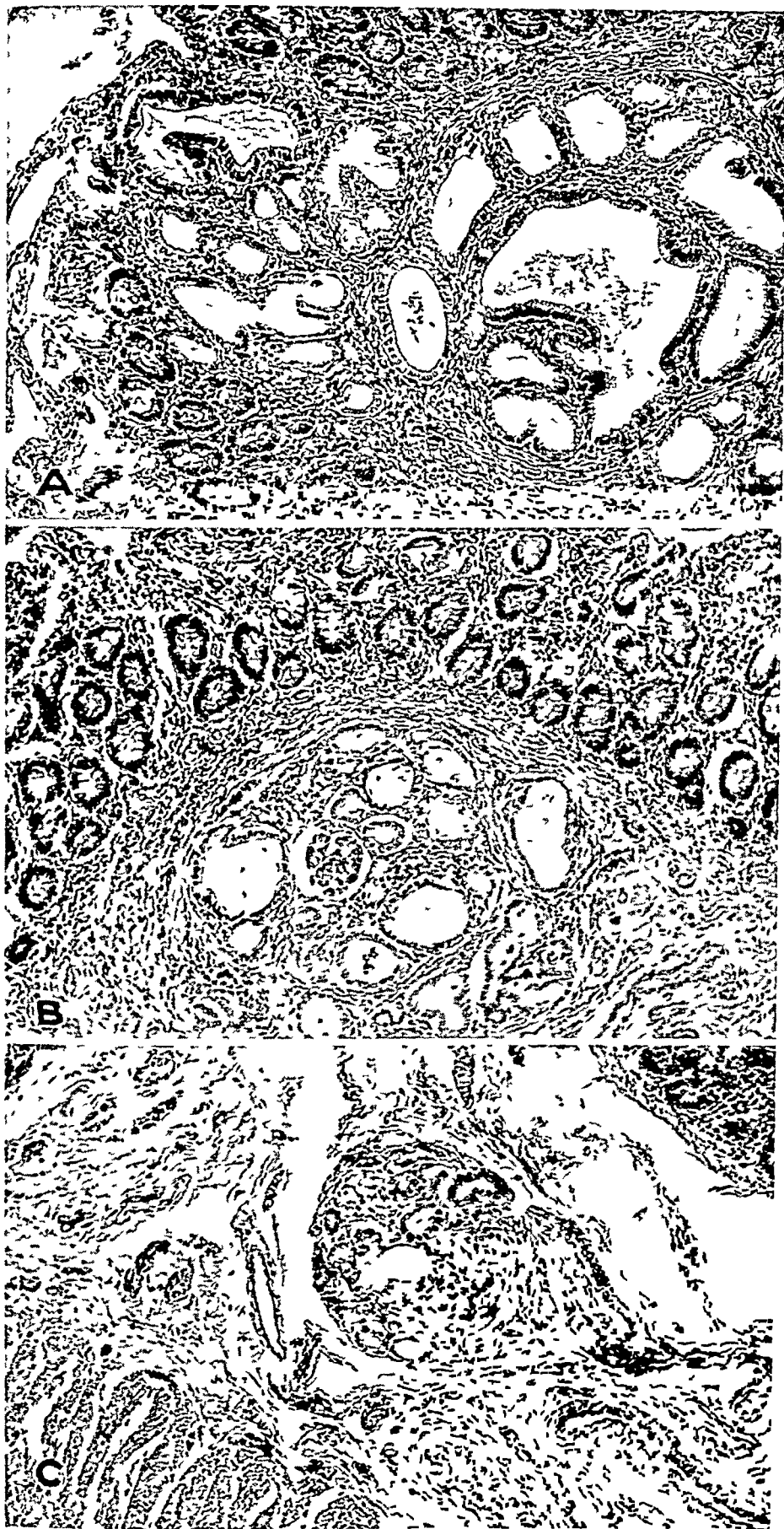


Fig 1—*A*, photomicrograph of a section of the orifice of Wirsung's duct as it enters the mucosa of the duodenum,  $\times 100$  *B*, Wirsung's duct in the submucosa, already greatly narrowed,  $\times 100$  *C*, Wirsung's duct just before its obliteration in the connective tissue of the submucosa  $\times 100$  The duodenal mucosa is just visible in the right upper corner, and the muscularis of the duodenum is seen in the lower left corner



consisted of a series of multilocular cysts which communicated and opened into the lumen of the duodenum but did not penetrate in the opposite direction through the muscularis of the duodenum. The lining epithelium was flattened, but there was no squamous metaplasia. Serial sections of the pancreas showed complete alteration of its structure (fig 2). It consisted of a series of large and small cysts containing a coagulum which in places was inspissated and even calcified. The cysts were embedded in a fibrous stroma. All of the cysts could be traced in the serial sections until they disappeared, and none extended through the tissue as would a duct, nor could any continuous lumen resembling a duct be found. The ducts and acini were so dilated as to be indistinguishable. No squamous metaplasia was found in the pancreatic tissue. Numerous islands of Langerhans of normal structure were seen in the fibrous stroma.

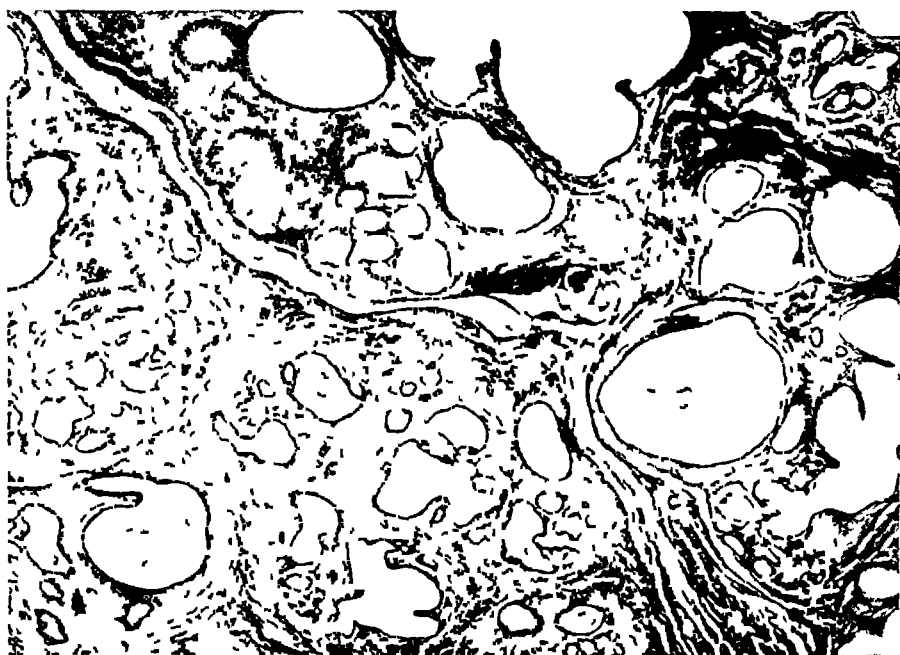


Fig 2—Photomicrograph of a section of pancreas showing irregular cystic dilatations embedded in dense connective tissue,  $\times 40$ . Some of the cysts contain coagulum.

*Final Anatomic Diagnosis*—Congenital atresia of the main pancreatic duct, cystic atrophy of the pancreas, vitamin A deficiency, squamous metaplasia of the trachea, bronchioles and salivary glands, purulent bronchitis, lobular pneumonia.

#### COMMENT

In this case of cystic fibrosis of the pancreas in a 10 month old baby, serial sections revealed the pancreatic lesion to be caused by true atresia of the pancreatic duct. This case is similar to that described by Tiling and Benoit. The pancreatic duct was lost in fibrous tissue before it penetrated through the muscularis of the duodenum. In the pancreas only dilated cysts, representing ducts and acini, were found, and none was connected to the duodenum. Fairly normal epithelium lined these cysts, so that this change could not be caused by occlusion of the pan-

creatic duct by squamous metaplasia. Quite the reverse had occurred. As the pancreatic duct was occluded, there were no pancreatic ferments in the duodenum, with resulting poor absorption of fats and a characteristic fatty condition of the liver. Anderson also noted this hepatic change in many of her cases. Furthermore, the poor absorption of fats had evidently been the basis for the vitamin A deficiency, i. e. the squamous metaplasia in the epithelium of the respiratory tract. Anderson also found this present in her cases and quoted Basu's earlier observation<sup>10</sup> that there is inadequate absorption of vitamin A from the alimentary tract if absorption of fat is inadequate. It is interesting that the bones of this child showed no rachitic change. In Anderson's cases, although vitamin A deficiency was a frequent finding, rickets was present only once. This indicates clearly that vitamin A is absorbed only with the fats absorbed from the intestinal tract, and in the absence of this source of the vitamin no vitamin A is acquired, whereas in the absence of intestinal intake of vitamin D a substituting factor, such as sunlight, may provide the vitamin to the body.

Blackfan and Wolbach<sup>11</sup> described terminal bronchitis and pneumonia secondary to the squamous metaplasia of the epithelium of the respiratory tract which occurs in infants suffering from vitamin A deficiency. Anderson noted that this was invariably the cause of death in her patients. In this infant, too, purulent bronchitis, bronchiolitis and pneumonia were the cause of death.

#### SUMMARY

Cystic fibrosis of the pancreas in an infant is reported. Serial microscopic sections showed that congenital atresia of the main pancreatic duct was the basis of the pancreatic lesion. Poor absorption of fats and deficiency of vitamin A resulted, and death occurred from pulmonary infection secondary to squamous metaplasia of the bronchial epithelium.

10 Basu, N. K. *Ztschr. f. Vitaminforsch.* **6** 106, 1937.

11 Blackfan, K. D., and Wolbach, S. B. *J. Pediat.* **3** 679, 1933.

# SPONTANEOUS DOUBLE RUPTURE OF THE HEART

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Spontaneous rupture of the heart is not rare<sup>1</sup> It has been recorded in about 0.0016 per cent of necropsies and is estimated<sup>2</sup> to occur in about 2 to 6 per cent of myocardial infarctions The anterior wall of the left ventricle is the commonest site of rupture The interventricular septum is rarely involved I have been unable to find a report of any case of septal rupture subsequent to the 26 cases mentioned in the survey by Benson, Hunter and Manlove,<sup>1c</sup> in 1933 These and other reports<sup>3</sup> make an example of double rupture of the heart seem unique

## REPORT OF A CASE

A man thought to be about 60 years old, with a history of old hemiplegia, convulsive seizures and inability to talk, was admitted to Western State Hospital, Dec 17, 1931 He was found to have spastic right hemiplegia, motor aphasia and "irregularity in the rhythm and force of the heart tones" The clinical diagnosis was "psychosis with organic brain disease (cerebral hemorrhage)" While in the hospital, he gradually deteriorated mentally and physically and had occasional convulsive seizures with transient cyanosis and irregularities of pulse Feb 11, 1939, he was found to have edema of the legs He suddenly became dyspneic and cyanotic, with a weak pulse Two days later he was found dead, fifteen minutes after having "seemed as usual"

Necropsy revealed an obese white man 176 cm in length, weighing about 210 pounds (95 Kg), with dependent edema The tense bluish pericardial sac was of triangular outline and measured 17.5 cm transversely Within it were about 200 cc of fluid blood and a soft red clot weighing 476 Gm The greatest trans-

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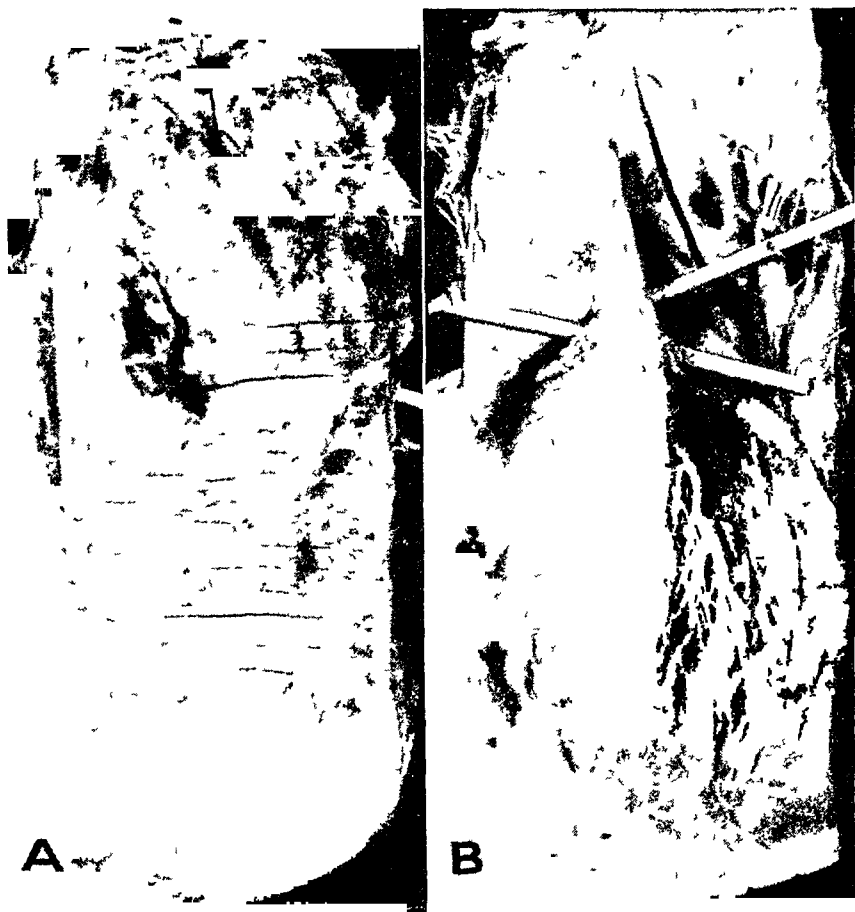
From the Department of Pathology, Western State Hospital, Fort Steilacoom, Wash

1 (a) Krumbhaar, E B, and Crowell, C Am J M Sc **170** 828, 1925 (b) de la Chapelle, C E Am Heart J **1** 315, 1926 (c) Benson, R L, Hunter, W C, and Manlove, C H Am J Path **9** 295, 1933 (d) Salzmann, H A Am J M Sc **188** 347, 1934 (e) Peterson, M C Minnesota Med **17** 256, 1934 (f) Clowe, G M, Kellert, E, and Gorham, L W Am Heart J **9** 324, 1934 (g) McNamara, F P, Hancock, J C, and Coady, C C J Iowa M Soc **27** 210, 1937

2 The Criteria Committee of the New York Heart Association Nomenclature and Criteria for Diagnosis of Diseases of the Heart, New York, J J Little and Ives Co, 1939

3 Davenport, A B Am J M Sc **176** 62, 1928 Stevenson, R R, and Turner, W J Bull Johns Hopkins Hosp **57** 235, 1935 Carter, D Y Brit M J **2** 335, 1936 McNamara, W L, Ducey, E F, and Baker, L A Am Heart J **13** 108, 1937

verse diameter of the anterior surface of the heart was 14 cm. There was extensive petechial and ecchymotic hemorrhage beneath the epicardium of all cardiac chambers posteriorly. The right ventricle was empty. The right auricular appendage was distended by adherent grayish thrombi, the left one was empty and contracted. On the posterior surface of the left ventricle, 1.5 cm to the left of the posterior interventricular sulcus and 3 cm from the atrioventricular sulcus (*A* in figure), was a ragged longitudinal laceration 2.2 cm long. The myocardium at its edges was soft, friable and yellowish. The heart weighed 570 Gm. The right coronary artery was considerably larger than the left, and its lumen was



*A*, posterior view of the septal areas of the ventricular wall showing the rupture through the posterior wall of the left ventricle. The bristle passes through the septal rupture. *B*, anterior view of a section through the septum and ventricles. The long bristle passes through the septal rupture which connects the two ventricles. The short bristle passes from the hemorrhagic cavity in the septum into the left ventricle. The internal opening of the rupture in the posterior left ventricular wall is just above the level of the long bristle, between the left edge of the septum and the knife cut which is visible in the posterior left ventricular wall.

unevenly distorted and narrowed by yellowish atheromatous plaques. Beginning at a point 15 mm from the right coronary ostium, the vessel was occluded for a distance of 20 mm by a dry, grayish and reddish thrombus. The artery passed

down the posterior interventricular sulcus as the posterior interventricular artery. The lumen of the left coronary artery, although tortuous and narrowed by atheromatous plaques with focal calcification, was patent throughout. Its circumflex branch turned downward and terminated over the posterior surface of the left ventricle just to the right of the obtuse margin without demonstrable anastomosis with the right coronary artery. The foramen ovale, though anatomically patent, was guarded by valvelike flaps adequate to close it. Both ventricles were dilated, and their papillary muscles and trabeculae carneae were thickened and flattened. The anterior wall of the right ventricle averaged 5 mm in thickness, the posterior wall was about 7 mm thick. Posteriorly, the muscular interventricular septum bulged into the right ventricle. The tricuspid valve was normal except for petechial hemorrhages on its posterior cusp, and the pulmonic valve was unchanged. The anterior wall of the left ventricle was elongated and measured 15 to 20 mm in thickness, while the posterior wall was 9 to 12 mm thick. Except for atheromatous plaques on the aortic cusps and on the anterior mitral leaflet, these valves were normal. There was extensive subendocardial hemorrhage of both left papillary muscles, over the posterolateral portions of the left ventricular wall and on both sides of the interventricular septum. The myocardium of the posterior wall of the left ventricle, the posterior papillary muscle, the upper posterior part of the septum and the posterior wall of the right ventricle were friable, softened, swollen, opaque and mottled reddish yellow and displayed tiny fibrotic foci and edematous areas. Elsewhere the muscle was coarsened, pinkish and firm. In the upper posterior part of the muscular septum (*B* in the figure) was a ragged tear, through which the ventricles communicated. The edges of this were covered by an adherent grayish red clot. On the right side, the septal rent measured 13 by 3 mm, on the left side it measured 12 by 8 mm. The opening sloped downward to the right and slightly forward. There was hemorrhagic dissection anteriorly into the septum in a plane parallel to the muscle fibers, and the cavity thus formed communicated with the opening into the left ventricle. The internal dimensions of the rupture in the posterior wall of the left ventricle were 10 by 4 mm. About this opening was a reddish clot.

Microscopic examination showed slight adventitial lymphocytic infiltration of the right coronary artery with marked atherosclerotic stenosis and occlusion of the lumen by a recently formed thrombus, in which there was variably complete disintegration of the blood cells but no evidence of organization. The thrombus in the right auricular appendage showed softening but no organization. Here some of the myocardial cells were swollen, and there was patchy interstitial edema with infiltration by leukocytes, chiefly polymorphonuclears. The myocardium of the anterior wall of the right ventricle was hyperemic and edematous and there was leukocytic exudate in the subepicardial fat. The anterior wall of the left ventricle and the anterior papillary muscle were modified by focal interstitial edema, slight, patchy fibrosis, focal infiltration by leukocytes and exudative inflammation of the subepicardial fat. The posterior right ventricular wall showed patches of young connective tissue containing lymphocytes and histiocytes and some necrobiotic changes in the muscle. The myocardium of the posterior left ventricular wall and of the corresponding papillary muscle exhibited focal recent fibrosis, edema, extensive coagulative necrosis, fresh hemorrhage and infiltration by well preserved leukocytes. About the septal rupture the muscle had undergone extensive coagulative necrosis and softening, with edema, hemorrhage and marked disintegration of the leukocytes and red cells of the abundant exudate. Here also were areas of vascular young granulation tissue containing old blood pigment, some of which was phagocytosed by histiocytes. All sections showed marked hypertrophy of the

muscle cells, which was greatest in those from the left ventricle. In some of the sections small intramural branches of the coronary arteries showed slight intimal fibrosis and atheromatous change.

Other important pathologic changes were marked generalized arteriosclerosis with multiple ulceration and mural thrombosis of the aorta, cerebral arteriosclerosis with multiple foci of cerebral, pontile and medullary softening, general chronic passive hyperemia with ascites, anasarca and bilateral hydrothorax with compression atelectasis of the congested and edematous lungs, bilateral renal arteriosclerosis without nephrosclerosis, nodular fibroadenomatous hyperplasia of the prostate gland, and purulent ethmoiditis and sphenoiditis. The thyroid and other endocrine glands were normal.

#### COMMENT

Although not of clinical record, the presence of arterial hypertension may be presumed because of the renal arteriosclerosis and the marked hypertrophy of the heart, predominantly left ventricular, without other demonstrable cause. This hypertension was no doubt an important cause of the two distinct ruptures through the same myocardial infarct. It would seem that the septal rupture probably occurred first, possibly two days before death, when the symptoms of acute cardiac distress appeared so abruptly in this person with failing circulation. The septal portion of the infarct was the older of the two foci, since a thrombus had formed over it. The massive tamponading intrapericardial hemorrhage would have been fatal before thrombosis could have occurred had the ruptures been simultaneous or had the mural rhexis occurred first. If this assumption is true, the present case is additionally interesting for the reason that solitary septal ruptures usually kill as suddenly as do mural ones. Anastomosis between the anterior and posterior interventricular arteries apparently spared the anterior apical septum from infarction, but the circumflex branch of the left coronary artery was apparently incapable of saving much of the left ventricle.

#### SUMMARY

A unique example of thrombotic occlusion of the right coronary artery with extensive myocardial infarction and rupture through the posterior part of the muscular interventricular septum, with subsequent separate rupture through the posterior wall of the left ventricle, resulting in fatal hemopericardium, is reported.

## ENDOMETRIAL SARCOMA

ROBERT FIENBERG, M D, WESTFIELD, MASS

Endometrial sarcoma has been recognized as a pathologic identity for a long time. In fact, Virchow<sup>1</sup> described the diffuse type of this neoplasm in 1865 and agreed that polypoid types might occur. During the latter part of the nineteenth and the early part of the twentieth century, numerous cases were reported. Many were included in compilations of cases of sarcoma of the uterus and were not published under the title of endometrial sarcoma. References to this older literature may be found in Piquand's<sup>2</sup> review of 1905 and in Ewing's<sup>3</sup> "Neoplastic Diseases."

Despite the voluminous early literature, endometrial sarcoma is today a little known entity. Stout,<sup>4</sup> for instance, stated that he has seen only 1 case. He believes that in many of the older reports cases of undifferentiated carcinoma were included among the cases of sarcoma.

Masson<sup>5</sup> compiled a series of 50 cases of uterine sarcoma that were observed during a period of twenty years and found only 1 case of endometrial sarcoma.

The difficulty in diagnosis may be responsible for the apparent rarity, with the result that cases of endometrial sarcoma are classified as cases of leiomyosarcoma. On the other hand, the frequency of reports of cases of endometrial sarcoma in the older literature may be due, as Ewing<sup>3</sup> said, to the fact that the cases were instances of advanced mural leiomyosarcoma.

R. Meyer<sup>6</sup> followed the teachings of Virchow and on the basis of gross anatomic features distinguished between a typical diffuse type of endometrial sarcoma and a polypoid type. He described the diffuse type as closely resembling histologically the lamina propria of the endometrium and, like it, possessing a typical reticulum, consisting of delicate fibrils surrounding individual cells. On the other hand, the polypoid forms are more akin to the mucosal polyps than to the lamina propria of the endometrium, possessing as they do a fibrillary stroma and spindle cells. Atypical and mixed cells are found in the latter type.

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From the Laboratory of Pathology, Westfield State Sanatorium

1 Virchow, R. Die krankhafte Geschwulste, Berlin, A. Hirschwald, 1864-1865, vol 2 (a) p 350, (b) p 180

2 Piquand, G. Rev de gynec et de chir abd 9 387, 1905

3 Ewing, J. Neoplastic Diseases, ed 3, Philadelphia, W. B. Saunders Company, 1929, p 284

4 Stout, A. P. Human Cancer, Philadelphia, Lea & Febiger, 1932, p 357

5 Masson, J. D. Am J Obst & Gynec 5 345, 1923

6 Meyer, R., in Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1930, vol 7, pt 1, p 374

Since 1918, scattered reports have appeared in the literature under the title of endometrial sarcoma. Brady,<sup>7</sup> Gerich,<sup>8</sup> Ward,<sup>9</sup> Offergeld,<sup>10</sup> Moench and Meeker<sup>11</sup> and Burger<sup>12</sup> have each published 1 case. Casler<sup>13</sup> described a "unique, diffuse, uterine tumor really an adenomyoma with stroma but no glands" in a woman 39 years of age. This was no doubt an endometrial sarcoma arising from the lamina propria of the endometrium.

Tudhope and Chisholm<sup>14</sup> described 3 cases which they thought might be mistaken for cases of endometrial sarcoma. Close examination of tissues routinely stained, as well as of those stained by Foot's modification of Bielschowsky's silver stain for reticulum, revealed, according to the authors, that all were instances of undifferentiated carcinoma.

My own experience has led me to believe that this neoplasm is not well known among pathologists and gynecologists. Certainly few reports have appeared in the literature recently. It is with these points in mind that the following 2 cases are reported.

#### REPORT OF CASES

**CASE 1**—A white woman 65 years old entered the cancer section of the Westfield State Sanatorium, Dec 9, 1937, because of intermittent vaginal bleeding since November 1937. The menopause had occurred at the age of 46. There was a spherical uterus, about 8 cm in diameter, with a fungating growth within it. On December 17, after dilation and curettage, 50 mg of radium was inserted into the body of the uterus, a total dose of 2,400 milligram hours was given. The pathologic diagnosis on the basis of the curettings was endometrial sarcoma. External irradiation was attempted but discontinued after the patient had received 600 roentgens to the pelvis on account of low red cell counts. Three transfusions were given, and a total hysterectomy was done.

The surgically removed specimen was a uterus amputated above the cervix, with tubes and ovaries attached. The cervix was submitted separately. The uterus measured 6 by 6.5 by 6 cm. The serosa was smooth. When the anterior wall was sectioned, a large, soft, yellowish brown, broad, lobulated polypoid mass was found attached to the posterior wall by a broad base, completely filling the endometrial cavity. It measured 8.5 by 9.5 by 2.5 cm, having expanded when the uterus was opened. On section it showed yellowish brown tissue with the consistency and appearance of cerebral tissue. Numerous regions of cystic degeneration were present. There were hemorrhagic foci on the surface. There was little invasion of the myometrium at the base on gross examination. The myometrium throughout the uterus, both at the base of the neoplasm and elsewhere, measured 1.5 cm in thickness. The endometrium covering the anterior wall was smooth and thin. A small leiomyoma, measuring 0.8 cm, was found just above the cervix in the anterior wall, and another of the same size, in the left cornu. Both the

7 Brady, L. *Bull Johns Hopkins Hosp* **29** 164, 1918

8 Gerich, O. *Zentralbl f Gynak* **53** 2016, 1929

9 Ward, C V. *Canad M A J* **25** 707, 1931

10 Offergeld, H. *Ztschr f Krebsforsch* **39** 191, 1933

11 Moench, G H, and Meeker, L H. *Am J Obst & Gynec* **30** 435, 1935

12 Burger, P. *Bull Soc d'obst et de gynec* **25** 274, 1936

13 Casler, DeW B. *Surg, Gynec & Obst* **31** 150, 1920

14 Tudhope, G R, and Chisholm, A E. *J Obst & Gynaec Brit Emp* **41** 708, 1934



ovaries and the tubes were normal. The cervix measured 3.5 by 2.0 by 2.5 cm. A patulous orifice with bilateral tears was present.

Section through the neoplasm revealed replacement of the endometrium by cellular, compact, homogeneous neoplastic tissue resembling to a striking degree the lamina propria of endometrium. No acini were visible. The neoplastic cells possessed small oval to round nuclei, containing fine chromatin granules. There was an occasional small nucleolus. There was little variation in the size and shape of the cells. There was scanty cytoplasm with indistinct cell outlines. A small amount of intercellular substance was present. There were numerous small arteries and veins with hyperplasia of the endothelium and mural cells. Numerous mitotic figures were seen. There was superficial, but definite, invasion of the myometrium. An intact layer of simple columnar epithelium was seen on the surface. No effect of irradiation was noted. With Wilder's reticulum stain, delicate reticular fibers formed a mesh resembling closely that found in the lamina propria of the endometrium. Reticular fibers were in intimate relationship with each cell, curving about and practically enclosing each one individually. These reticular fibers were much thinner than those in the myometrium.

The rest of the endometrium was atrophic. Small, ill defined nodules of criss-crossing bundles of smooth muscle formed small leiomyomas within the myometrium. There was moderate lymphocytic infiltration of the cervical stroma, with few dilated glands visible. The ovaries and tubes were normal.

The diagnosis was endometrial sarcoma, leiomyoma of the uterine fundus and chronic cervicitis.

The patient died Jan. 29, 1938, with signs of cardiac failure.

*Laboratory Data*—Dec. 10, 1937, the red cell count was 1,420,000, the hemoglobin content 22 Gm (Sahli), the white cell count, 5,900. Jan. 22, 1938, the red cell count was 3,850,000, the hemoglobin, 10.2 Gm (Sahli), the white cell count, 8,400.

*Postmortem Examination*—The body was that of an elderly white woman, slightly emaciated. The abdomen was distended. A recent abdominal incision, 17 cm in length, held together by stay sutures, extended from a point at the left of the umbilicus to just above the pubis. The edges adhered well. A drain was present in the lower portion. The pelvis contained 25 cc of fresh blood. The duodenum and the loops of the jejunum were distended. There was slight distention of the cecum. A defect in the pelvic floor caused by removal of the internal genitalia was well repaired, with sutures intact.

The heart weighed 430 Gm. There was dilatation of the right ventricle and right auricle. The left ventricle was moderately enlarged and the left ventricular wall thickened. On section, the myocardium of the left ventricle displayed a few yellowish white foci of fibrosis, measuring up to 0.5 cm in diameter. There was a slight amount of fibrosis in the rings of the aortic and pulmonic valves.

The left lung weighed 300, the right 420, Gm. There was congestion of the posterior portions of the upper lobes, with atelectasis of the posterior portions of both lower lobes. The bronchi contained thick mucopurulent material. The main branches of the pulmonary artery were dilated, the secondary branches, within the lungs, were normal.

The left kidney weighed 240 Gm, the right, 228. The capsules stripped with difficulty, exposing a somewhat irregular, reddish brown surface with scattered thin-walled cysts, measuring from 0.7 to 5.5 cm in diameter. On section, the cortices were found markedly and irregularly narrowed, measuring 0.2 to 0.4 cm in thickness. The demarcation between the cortex and the medulla was poor on the right and on the left. An encroachment of cysts on the medulla was visible

The thyroid weighed 45 Gm. The left lobe contained a few small nodules measuring up to 3.5 cm in diameter. The nodules were well encapsulated, translucent, yellowish. In the right lobe were similar but smaller nodules, measuring up to 1 cm in diameter.

The left sacroiliac joint and vertebrae were examined, but no metastatic tissue was found.

The head was not examined.

*Microscopic Examination*—Sections were stained with phloxine-methylene blue. In the heart were small patches of fibrosis and thickening of the intima of small arteries, in the lungs, marked congestion and hemorrhage. The kidneys showed numerous wedge-shaped cortical scars with scattered lymphocytes, many cortical and medullary cysts with thin, fibrous walls, lined with flattened epithelial cells, and thickening of the intima of medium-sized arteries. An occasional hyalinized arteriole could be seen. There was lymphocytic infiltration of the submucosa of the pelvis.

The aorta showed fibrous thickening of the intima with cholesterol deposits.

There was marked fibrosis in the nodules in the thyroid, with dilated acini, filled with colloid. There was much colloid in the connective tissue between the acini.

Bone from the iliosacral joint and vertebrae was normal. No metastases were observed.

The main postmortem diagnoses were panhysterectomy for sarcoma of the endometrium, hypertensive cardiac hypertrophy and paralytic ileus.

**CASE 2**—A white woman 72 years old was admitted to the cancer section of the Westfield State Sanatorium Oct 4, 1938. She had had vaginal bleeding since June 1937. There was a foul vaginal discharge. There had been dull aching pain in both lower quadrants of the abdomen for the past two months, also, slight frequency of urination. The loss of weight was 8 pounds (3.6 Kg). The menopause occurred at the age of 55.

Physical examination revealed a large, hard tumor in the lower quadrant of the abdomen, reaching the umbilicus. A hard, nodular, freely bleeding mass was felt in the cervical canal. The uterus was freely movable.

October 7, after dilatation and curettage, a specimen was taken from the cervix for biopsy, and 100 mg of radium in a platinum bomb was inserted. The patient received 1,700 milligram-hours of radium exposure. The pathologic diagnosis on the curettings was chronic cervicitis with necrosis of tissue. The patient was discharged against advice October 17 but was admitted a second time Jan 20, 1939. Vaginal bleeding had continued. The pelvic condition was similar to that on first examination. Exploratory laparotomy was done January 30. A large uterus was found with an irregular nodule on the superior surface of the fundus, with two loops of small intestine attached. On account of inoperability, biopsy alone was done. The pathologic diagnosis on the tissue removed was "sarcoma, insufficient for classification." The patient died February 8.

*Laboratory Data*—Jan 5, 1938, the red cell count was 4,330,000, the hemoglobin content, 11.3 Gm (Sahli), the white cell count, 6,300. February the red cell count was 3,300,000, the hemoglobin, 9.9 Gm (Sahli), the white cell count, 20,200.

*Postmortem Examination*—The body was that of an obese white elderly woman. The abdomen was markedly distended. A recent, poorly healed surgical incision extended from just above and to the right of the umbilicus down toward the suprapubic region for about 20 cm. There was an abundant amount of foul-smelling grayish purulent material in the vaginal orifice. The mucosa of the lower portion of the vagina was ulcerated and grayish green. The large and small intestines

were distended. Loops of small intestines were adherent with friable grayish fibrinous adhesions. About 600 cc of a grayish brown purulent fluid was distributed generally. The enlarged uterus protruded from the pelvic cavity with a grayish black, mottled nodule on the superior aspect of the fundus. Two loops of proximal ileum were adherent to the nodule. The peritoneum was thickened and discolored grayish green to grayish black, especially on the right. The perineal fat was also thickened. There were abundant fibrinous adhesions between the right lobe of the liver and the right dome of the diaphragm. The sigmoid was compressed by the enlarged uterus and was adherent to the left lateral gutter by means of dense fibrous adhesions. The pouch of Douglas was obliterated. The omentum was moderately contracted, thickened and indurated, with an abundant amount of fat present. It revealed indistinct yellowish white striations.

In the pleural cavities there were dense fibrous adhesions at the apices of the left and right lungs. On the right 50 cc of straw-colored fluid was present. There were scattered yellowish plaques with slight calcification in the descending branch of the left coronary artery just below its junction with the left circumflex artery. In the lower lobe of the left lung there were superficial regions of atelectasis. On the right, atelectasis was more extensive, with half of the lower lobe involved.

The gastrointestinal tract was normal, but there was moderate induration of the perirectal tissues by fibrosis, with a small yellowish white nodule, about 0.7 cm in diameter, in the anterior portion of the perirectal tissues. The distal portion of the gallbladder was fibrotic and narrowed and enclosed two pigment-cholesterol stones. There were small pigment stones in the neck. The common duct was dilated, 1.4 cm in diameter. The kidneys each weighed 160 Gm. The cortices were well demarcated from the medullae but were narrowed, measuring 0.3 to 0.4 cm in thickness. There was a small cyst with purplish fluid in the upper pole of the left kidney. In the bladder, in the region of the trigon, were small, slightly translucent, reddish brown excrescences, 0.1 cm in diameter.

The fundus of the uterus was much enlarged, measuring 13 by 9.5 by 9.5 cm. The cervix measured 1.5 cm in length. The upper portion of the anterior surface of the fundus was smooth, the lower portion was adherent to the bladder by fibrous and fibrinous adhesions. The anterior wall of the uterus was sectioned, revealing a dilated endometrial cavity, measuring 18 cm in circumference. It was filled with large, necrotic, friable, yellowish red to yellowish white polypoid masses with grayish green to grayish black tips. The entire endometrial surface was involved by the neoplasm except for the lower segment, measuring 3.5 cm in length from the lower edge of the neoplasm to the internal cervical orifice. On section the neoplastic tissue possessed a variegated color pattern corresponding to the colors of the surface. Grossly, the uterine wall appeared more compressed than invaded, with only a thin rim of myometrium present, measuring from 0.2 to 1.5 cm in thickness. The uterine wall of the superior portion of the fundus, where the nodule was found attached to loops of proximal ileum, was grayish green, necrotic and very thin, measuring about 0.2 cm in thickness. The exocervix was flattened. The cervix itself was uninvolved by the neoplasm. The pericervical tissues were indurated and fibrotic. The entire vagina was ulcerated and grayish green. There was infiltration of the left broad ligament by the neoplasm, there was thickening of the right broad ligament, with no neoplastic infiltration. The left fallopian tube was slightly dilated, 0.7 cm in diameter. The fimbriated end was obliterated and adherent to the cystic left ovary, which measured 1.5 cm in diameter. There were flecks of fibrin on the surface of the left ovary. The right tube was more dilated, measuring 1.2 cm in diameter. The surface was grayish green and covered with

flecks of fibrin. The fimbriated end was obliterated and adherent to the right ovary, which measured about 1.2 cm in diameter.

The aorta showed scattered yellowish plaques, with early calcification present, especially in the lumbar region.

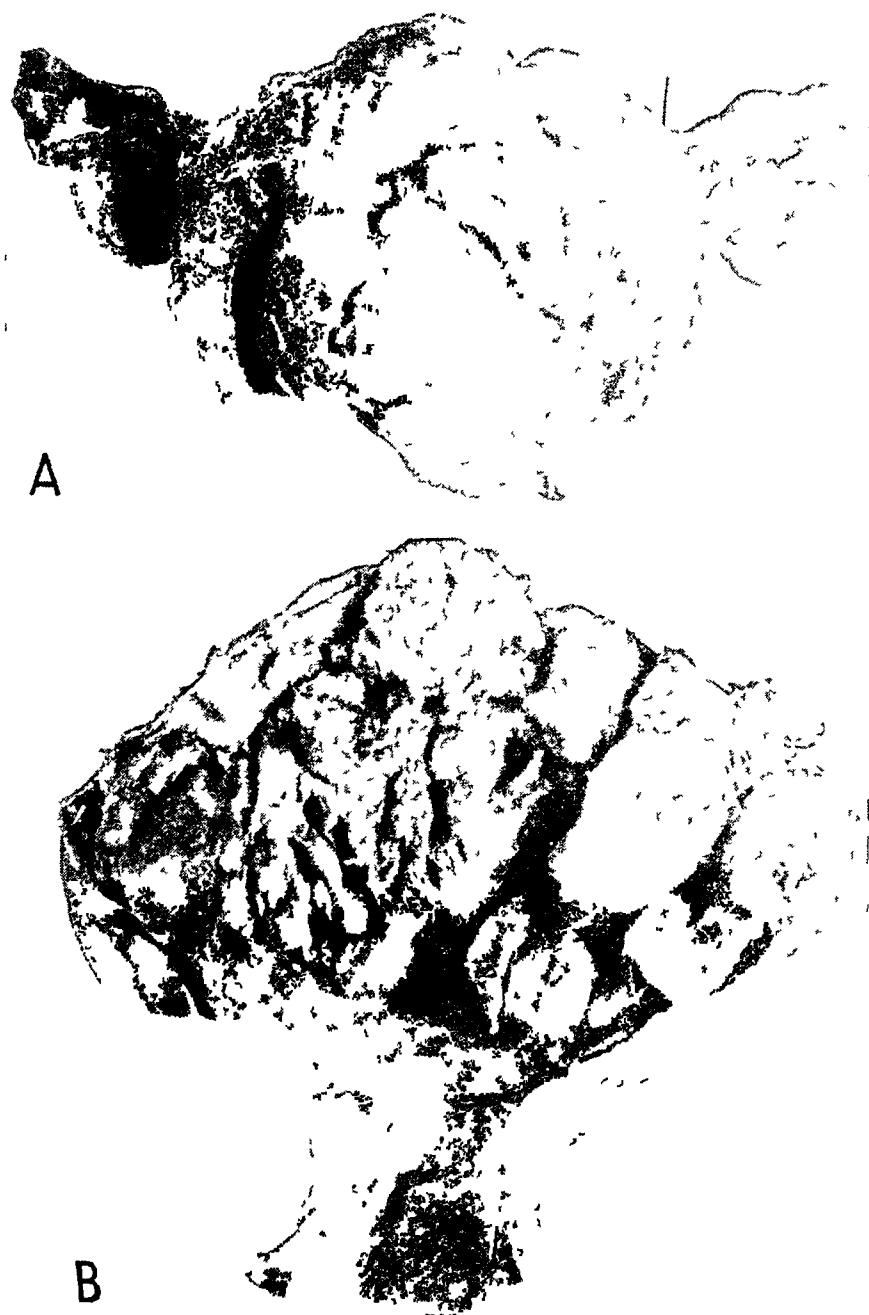


Fig 1—*A*, uterus with an endometrial sarcoma having its origin in the lamina propria (case 1). *B*, uterus with an endometrial sarcoma having its origin in an endometrial polyp (case 2).

The head was not examined.

*Streptococcus haemolyticus* (beta) was isolated from the peritoneal exudate after death.

## EXPLANATION OF FIGURE 2

*A*, section of the endometrial sarcoma in case 1, hematoxylin-phloxine,  $\times 430$   
Note the homogeneity of the cells and their similarity to those of the lamina propria of the endometrium

*B*, delicate mesh of fibers similar to that found in the lamina propria of the endometrium enclosing the individual sarcomatous cells (case 1), Wilder's reticulum stain  $\times 430$

*C*, section of a rapidly growing, immature portion of the sarcomatous uterine neoplasm in case 2, showing a streaming bundle of characteristic sarcomatous spindle cells with mitotic figures, phloxine-methylene blue,  $\times 430$

*D*, section of a slowly growing, mature portion of the uterine neoplasm in case 2, showing masses of carcinoma surrounded by sarcoma, phloxine-methylene blue,  $\times 100$

*E*, section of a slowly growing, mature portion of the sarcomatous uterine neoplasm in case 2, showing coarse, slightly wavy long fibers running parallel to the long axes of sarcomatous cells, Wilder's reticulum stain,  $\times 430$

*F*, section of a carcinomatous metastasis in perirectal tissues showing masses of carcinoma separated from sarcoma by a thin zone of acellular connective tissue (case 2), phloxine-methylene blue,  $\times 100$

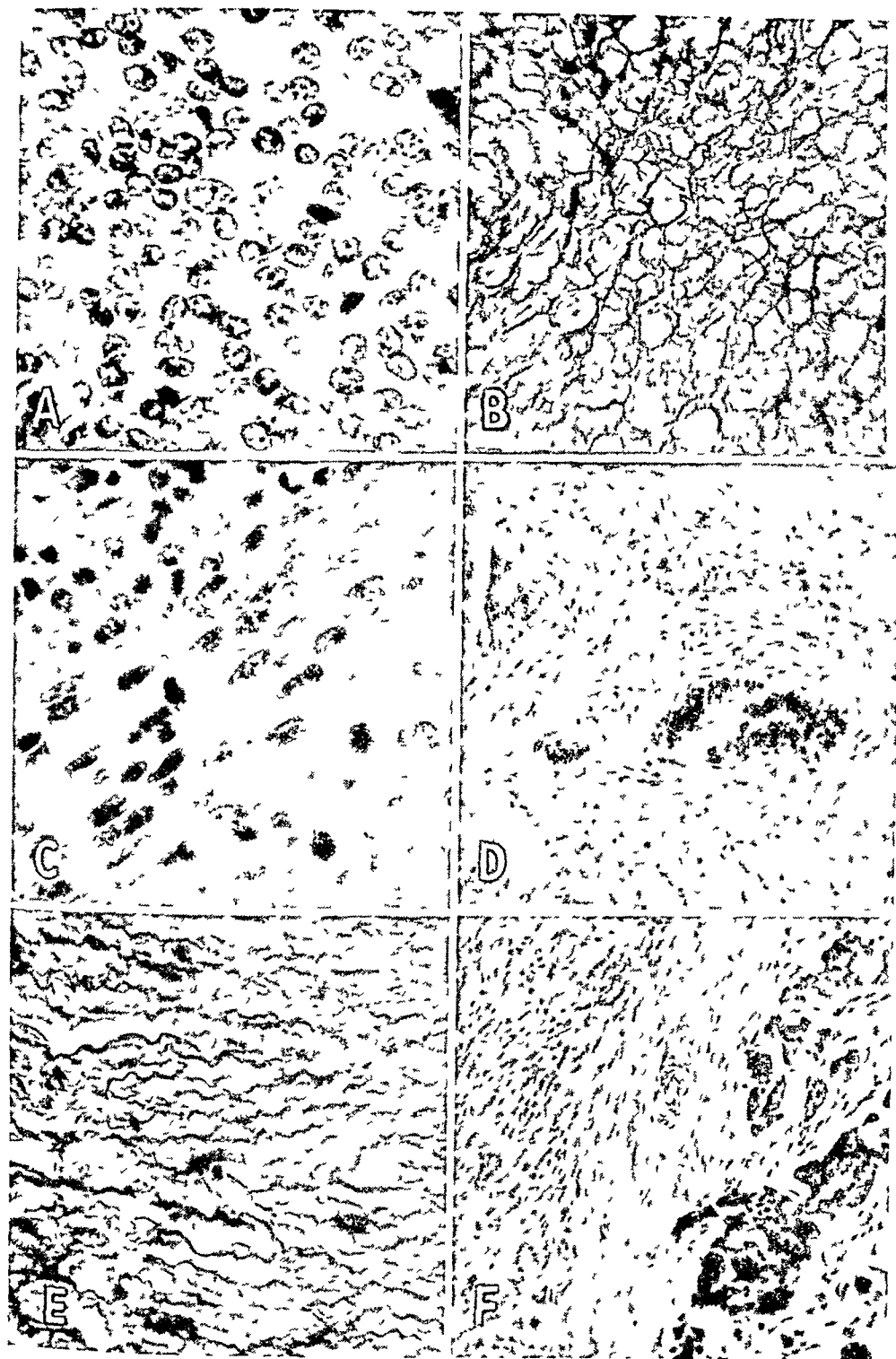


Figure 2

*Microscopic Observations*—Sections were stained with phloxine-methylene blue, Masson's trichrome stain as modified by Goldner<sup>15</sup> and Wilder's stain for reticulum. The lungs and spleen were congested. There was marked edema, with deposition of fibrin, in the submucosa of the small intestine. There was thickening of the serosa by edema and fibroblastic proliferation with thick layers of fibrin and neutrophils on the surface. The rectum was normal, but small masses of compact, polygonal neoplastic epithelial cells were present within the perirectal tissues. The oval and round nuclei of the neoplastic cells contained coarse chromatic granules and occasional large nucleoli. There were scattered giant lobulated nuclei. An occasional mitotic figure was seen. The cytoplasm was acidophil and moderately abundant. There was infrequent acinus formation. There was edematous stromal connective tissue about the neoplastic epithelial masses, the former sharply differentiated from the latter. Thin stromal trabeculae separated the neoplastic epithelial masses from one another. The stroma was predominantly cellular, small vesicular spindle-shaped nuclei were present in most places, but no mitotic figures. There was little variation in size or shape. In one region, however, the stroma was definitely sarcomatous. Here, an acellular zone of connective tissue was present about the neoplastic epithelial masses, with sarcomatous tissue beyond. There was transition between the benign cellular connective tissue stroma and the sarcomatous tissue. The latter was arranged in criss-crossing bundles, with oval and spindle-shaped nuclei, which were definitely plumper and more hyperchromatic than those in non-neoplastic stroma. The nuclear chromatin material was arranged in fine granules, with occasional small nucleoli present. The cytoplasm was scanty. Numerous mitotic figures with atypical forms were present. There was moderate variation in size. The phloxine-methylene blue stain revealed a moderate amount of intercellular material. With Wilder's stain for reticulum, the masses of carcinoma were found to contain no silver-staining fibers, but the stromal reticulum about the carcinomatous masses was prominent. Within sarcomatous stromal portions the reticulum stood out sharply, with numerous thick and thin, long, slightly wavy, at times reduplicated silver-stained fibers noted in intimate contact with sarcomatous cells. This reticulum was similar to that found in more mature portions of the uterine neoplasm, to be described later.

A few small foci of fat-filled cells were noted in the liver. The wall of the gallbladder was thickened and fibrotic, with large foci of cholesterol and small foci of calcification. Scattered lymphocytes were noted. The serosa was edematous and infiltrated by neutrophils, lymphocytes and endothelial cells. One section of a kidney had a cyst between the cortex and the medulla, with a narrow zone of fibrous renal parenchyma at the periphery. There were wedge-shaped scars in the cortex. The lamina propria of the bladder showed moderate edema and congestion, with foci of lymphocytes scattered about. In one section there was invasion of the serosa by masses of neoplastic epithelial cells similar to those found in the perirectal tissue, but no sarcomatous tissue was present. Numerous sections through the uterine neoplasm revealed a predominantly rapidly growing, ulcerated, solid sarcomatous tissue arranged in criss-crossing bundles with a herringbone pattern visible. There were scattered thin-walled vessels. The cells were typically fibroblastic. The nuclei were predominantly spindle shaped with some oval, round and giant lobulated nuclei visible. They contained fine chromatin granules and round, prominent, single or multiple nucleoli. There were numerous mitotic figures. The cytoplasm was granular, collected at the poles and tapered to fine points.

15 Goldner, J. *Am J Path* 14 237, 1938

There was much necrosis. The nonulcerated portions were covered by simple columnar epithelium. There was marked invasion of myometrium, with isolated foci of muscle surrounded by sarcomatous tissue. There was a thin layer of myometrium at the periphery in some sections. There was invasion of veins by the neoplasm. There was organizing fibrin on the serosa. In this rapidly growing sarcomatous tissue, occasional coarse intercellular fibrils were visible with phloxine-methylene blue stain. With the trichrome stain, a few scattered intercellular collagen fibers were noted. Wilder's stain revealed a few coarse, long, slightly wavy reticular fibers running along the long axis of the cells.

In two sections the neoplasm was more mature and slowly growing. The surface was covered by simple columnar epithelium, occasionally indented, forming acini. Occasional small solid epithelial masses were also present. Within acini the epithelial cells were often arranged in several layers. The epithelial nuclei were oval to round with occasional definitely neoplastic, multinucleated and multilobular giant cells. The cytoplasm was scanty and acidophilic, with occasional round acidophilic cytoplasmic inclusion bodies present. Papillary projections within acini were composed of sarcomatous tissue covered by epithelial cells. The trichrome stain revealed much more intercellular collagen here than in rapidly growing portions. The tissue did not stain like smooth muscle. Wilder's stain for reticulum revealed numerous coarse, long, slightly wavy fibrils along the long axes of cells in intimate contact with them. These fibers were similar to those in the stromal sarcoma of the perirectal tissues. No irradiation effect was noted in the uterine neoplasm. There were numerous lymphocytes and polymorphonuclears within the mucosal folds and in the lumens of the tubes. In one section of ovary there were small carcinomatous masses at the periphery, similar to those in the perirectal tissues. The stroma about the carcinomatous masses was also similar to that in the perirectal tissues except for absence of sarcomatous change. A small focus of sarcoma was noted at some distance from the carcinomatous masses, with moderate polymorphism, similar to that seen in rapidly growing portions of the uterine sarcoma. The thyroid contained a small nodule composed of poorly developed acini. Many epithelial cells had vacuolated cytoplasm. There was marked necrosis of the surface of the abdominal wound with numerous neutrophils and lymphocytes below. No fibroblastic activity was seen. There were occasional foci of cellular marrow in an otherwise atrophic marrow in the vertebrae.

The main postmortem diagnoses were endometrial sarcoma of the uterus with foci of carcinoma and local extension and metastases to the perirectal tissues, bladder and ovary, acute fibrinopurulent peritonitis.

#### COMMENT

The 2 cases described are offered as instances of the two types of endometrial sarcoma.

In the first the tumor, except for its uniradicular, polypoid appearance, was typical of sarcoma arising from the endometrial lamina propria. Consistent with this origin were the location of the neoplasm and the superficial invasion of the myometrium. In addition, not only did the characteristics of the cells stained with hematoxylin and phloxine leave no doubt of its histogenesis but the reticulum within the neoplasm was characteristic, simulating as it did the reticulum of the endometrial lamina propria. This reticulum was delicate and consisted of fibers which twisted and turned, enclosing each cell individually. This pattern was in sharp contrast to the long, thick reticular fibers in leiomyosar-



coma, which extend parallel to the long axes of the cells. Whether this type of endometrial sarcoma grows in an undifferentiated form and thus loses its reticulum could not be ascertained from the literature.

Although the importance of the reticulum in diagnosing this type of neoplasm has been stressed by R. Meyer,<sup>6</sup> none of the articles describing this type of neoplasm published within the past twenty years has mentioned the use of a silver reticulum stain. It is true that Tudhope and Chisholm<sup>14</sup> used a silver reticulum stain, but they were concerned with differentiating carcinoma from sarcoma, and furthermore they had no neoplasm similar to that in the first case reported here.

No metastases were found at postmortem examination, which was consistent with the histologic appearance.

The absence of any irradiation effect on this neoplasm is striking. The 600 roentgen of radiation may be disregarded, but the 2,400 milligram hours of radium exposure given over a month before the total hysterectomy must be taken into consideration despite the bulk of the neoplasm. It appears that, as in leiomyosarcoma, radical surgical operation is the only rational treatment.

The neoplasm in the second case, which is similar to one cited by Piquand,<sup>2</sup> falls into the category of the classic polypoid type of endometrial sarcoma arising from polyp. This is a logical classification, since the stroma of the endometrium gave rise to the stroma of the polyp which in turn gave rise to the sarcoma.

In the neoplasm described, elements related to those of a fibrous polyp were noted in the more slowly growing portions of the neoplasm, with carcinomatous glandular structures intermingled with the sarcomatous stroma. The sarcomatous cells, unlike those in the neoplasm of the first case, were more spindle in type.

The reticulum in this neoplasm resembled that in endometrial polyp, with long, thick fibers running parallel to the long axes of the cells. The reticulum alone is not of as much importance as in the first case, since a similar type can be seen in leiomyosarcoma, but it is valuable if used in conjunction with the general histologic picture.

Of course, the fibroblastic characteristics of the cells and the abundant amount of intercellular collagen in intimate relationship to the cells in the mature portions showed that this neoplasm was not leiomyosarcoma.

In addition, the sarcomatous elements of the uterine neoplasm were definitely not undifferentiated carcinoma, since the reticulum, especially that in the slowly growing portions, was abundant and in intimate contact with the cells. Furthermore, the reticulum separated sharply the carcinomatous from the sarcomatous elements. It would be contrary to all histologic criteria to believe that a carcinoma could produce such typical sarcomatous reticulum. The few reticular fibers in the rapidly growing portion were a sign of poor differentiation.

The carcinomatous elements formed histologically only a small part of the uterine neoplasm. In fact, they were absent in the rapidly growing portions. Clinically, they were important because they were capable of metastasizing.

Of great interest is the stroma of the carcinomatous metastases, since definite transformation of the stroma into sarcoma took place. It is

doubtful on histologic grounds that this change represented metastasis from the uterine sarcoma. Furthermore, simultaneous metastases of both the carcinomatous and the sarcomatous elements to an identical spot, with the latter surrounding the former, would be extraordinary. A third point against metastasis from the uterine sarcoma is the fact that transition from the benign to the sarcomatous stroma could be traced.

The last point is of interest, since it indicates an influence of the carcinomatous element on its stroma, which led to malignant changes. Of course, if this was so in the metastatic foci, it seems that the same might hold true for the primary uterine neoplasm, where the influence by the carcinomatous elements on the stroma of the original polyp might have given rise to the sarcoma which later became the predominant neoplasm.

It is of interest that this idea of a malignant neoplasm possibly influencing adjacent non-neoplastic tissue toward malignant neoplasia was mentioned by Virchow,<sup>16</sup> who, however, expressed the opinion that the sarcoma preceded the carcinoma in cases similar to my second case. On the other hand, Ehrlich and Apolant,<sup>16</sup> who were the first to demonstrate such a phenomenon in animal neoplasms, were convinced that the carcinoma arose first and influenced its stroma to become sarcoma, which in turn became the predominant neoplasm. Herxheimer<sup>17</sup> and Harvey and Hamilton<sup>18</sup> agreed with Ehrlich and Apolant, they applied this idea to human neoplasms.

As in the uterine neoplasm, so in the metastatic foci, the sarcomatous elements could not be confused with undifferentiated carcinoma since a typical sarcomatous reticulum was found in the sarcomatous stroma, sharply differentiating them from the carcinomatous elements, which contained no reticulum.

No irradiation effect could be found on this neoplasm despite the application of 1,700 milligram-hours of radium exposure four months before death, although the bulk of the neoplasm, as in the first case, might have prevented easy access of the radium to the entire mass of the neoplasm. Radical surgical intervention would thus seem to be the best method of treatment for this type of neoplasm.

While I am aware that the second case might be regarded as an instance of carcinosarcoma, a discussion of this much debated subject (Saphir and Vass<sup>19</sup>) has been deliberately avoided to prevent this paper from becoming too diffuse and cumbersome.

#### SUMMARY

Of the 2 cases of endometrial sarcoma described, the first belongs to the group in which the neoplasm arises from the lamina propria of the endometrium, the second to the group in which it comes indirectly from the lamina propria by way of an endometrial polyp.

16 Ehrlich, P., and Apolant, H. *Centralbl f allg Path u path Anat* **17** 513, 1906, *Berl klin Wchnschr* **44** 1399, 1907.

17 Herxheimer, G. *Beitr z path Anat u z allg Path* **44** 150, 1908, *Centralbl f allg Path u path Anat* **29** 1, 1918.

18 Harvey, W. F., and Hamilton, T. D. *Edinburgh M J* **42** 337, 1935.

19 Saphir, O., and Vass, A. *Am J Cancer* **33** 331, 1938.

Within the predominantly sarcomatous uterine neoplasm of the second case were foci of carcinoma, which gave rise to metastases. The latter were surrounded by a cellular stroma which in one region was sarcomatous. This observation suggested that both the carcinoma in the uterus and that in the metastases might have influenced the stroma to become sarcoma, which in the uterus became the predominant neoplasm.

The value of reticulum staining to bring out the characteristic reticulum in endometrial sarcoma arising from the lamina propria has been stressed. The reticulum in the endometrial sarcoma arising from an endometrial polyp was found to be less characteristic. Reticulum staining was also of value in confirming the diagnosis of sarcoma in the stroma of the carcinomatous metastases of the second case and in distinguishing the sarcoma from undifferentiated carcinoma.

# Laboratory Methods and Technical Notes

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## HAMDI'S PRESERVING SOLUTION

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My teacher, the late Prof H Hamdi, used an inexpensive fluid for the preservation of gross pathologic specimens, which has been in general use in our country for some years. The simplicity, low cost and efficacy of the method lead me to describe it here.

The specimen to be preserved is fixed in a 10 to 20 per cent dilution of the 40 per cent stock solution on the market. The larger and more solid the specimen (brain, liver, large spleen, large tumor) the higher the percentage of stock solution that may be used. After the specimen has been thoroughly fixed and prepared, it is left in running tap water for at least twenty-four hours, then it is placed in a highly hypertonic (about 50 per cent) salt solution prepared with clean tap water. Here it is left for two to five days. The hypertonic salt solution penetrates the organ, drives out the formaldehyde solution, causes a slight swelling, which removes the shrinkage due to the formaldehyde fixation, and increases the weight of the specimen, so that the lungs, for instance, do not float when placed in the preserving fluid. It helps, moreover, to prevent the growth of molds. After the organ has been rinsed in running water, it is placed directly into Hamdi's solution, the formula of which is as follows:

Sodium sulfate (pure)	5 Gm
Salt (pure)	100 Gm
Clear tap water containing no organic impurities	1,000 Gm
Glycerin	50 Gm

The solution is clear, practically colorless. It may be yellowish if the glycerin is yellowish. Icteric organs sometimes give it an icteric tint. After a couple of years it may become slightly yellowish, especially when the specimen is exposed to much light.

A few drops of a saturated camphor solution in 96 per cent alcohol is added. The white precipitate that forms is dissipated by superficial stirring with a glass rod. Then the glass cover is sealed on the container.

If the seal loosens and molds form, and even if the color of the specimen is spoiled on account of the molds, it is sufficient to leave the specimen for half an hour to a few hours in running water and in a jar into which a few crystals of potassium permanganate have been placed. The organ is then rinsed in running water, treated with salt water or not, replaced in fresh Hamdi solution and the jar resealed.

The advantages of Hamdi's solution are. It does not necessitate a preliminary treatment of specimens with alcohol as with most of the preserving solutions now in use. It preserves the specimens practically in their natural condition, it does not destroy the red blood corpuscles.

### SUMMARY

An economical method of preserving pathologic and anatomic museum specimens is described.

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From the Laboratory for Pathological Anatomy, Gureba Hospital

# General Reviews

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## EXPERIMENTAL GASTRIC CARCINOMA

A CRITICAL REVIEW WITH COMMENTS ON THE CRITERIA  
OF INDUCED MALIGNANCY

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AND

WALTER LINCOLN PALMER, M.D.

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The production of experimental cancers is now generally considered to have started in 1914 with the finding of Yamagiwa and Ichikawa that repeated applications of coal tar to a rabbit's ear was followed in some cases by carcinomatous changes with metastasis. Since that time many different neoplasms have been produced experimentally by various methods. The studies of Kennaway, Cook, their co-workers and others in the isolation of a carcinogenic hydrocarbon from tar and later in the preparation and synthesis of other carcinogenic compounds opened up new methods of approach. In view of the rapid strides made in the past twenty-five years in the field of experimental cancer, it seems of interest to review what success has attended efforts to produce experimentally one of the most common malignant growths found in man—carcinoma of the stomach.<sup>1</sup>

In animals, carcinoma of the stomach is one of the rarest spontaneous cancers. Feldman<sup>2</sup> estimates that 8 to 10 per cent of old dogs have neoplasms, of which 40 to 50 per cent are malignant, yet all studies of the occurrence of spontaneous cancers in domestic, laboratory and wild animals uniformly show that carcinoma of the stomach is rare (Wells, Slye and Holmes<sup>3</sup>, Slye, Holmes and Wells<sup>4</sup>). There is no strain of animal in which gastric cancer develops regularly or indeed with sufficient frequency to warrant an attempt to develop a "cancer strain". Furthermore, the diagnosis for animals is almost invariably made post mortem.

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This review is preliminary to work aided by a grant from the Fenger Memorial Fund

1 References to some work, particularly that pertaining to parasitic tumors, are chiefly to key articles. If the same paper has been published in several languages or journals, only one reference is usually made to it.

2 Feldman, W. H. *Neoplasms of Domesticated Animals*, Philadelphia, W. B. Saunders Company, 1932, p. 68.

3 Wells, G. H., Slye, M., and Holmes, H. F. *Am. J. Cancer* **33**: 223, 1938.

4 Slye, M., Holmes, H. F., and Wells, H. G. *J. Cancer Research* **2**: 401, 1917.

In over 142,000 mice of the Slye stock, dying of natural causes, Wells, Slye and Holmes<sup>3</sup> reported 12 gastric cancers—8 squamous cell carcinomas, 3 adenocarcinomas and 1 sarcoma. In addition there were 2 adenomas which may have been early adenocarcinomas. This represents all the gastric cancers in the Slye stock of mice in over twenty years of careful observation. This report, essentially confirmed by all other investigators, stresses the rarity of spontaneous gastric neoplasms in mice.

Stewart and Andervont<sup>5a</sup> found that in mice of both sexes of strain I there regularly develop spontaneous adenomatous, hypertrophic and hyperplastic overgrowths of the glandular rugae of the pyloric portion of the stomach with associated inflammatory changes and ulceration, metastases have not been observed. Histologic studies revealed some features somewhat suggestive of malignant growth. A few adenomatous tumors in the lungs were regarded as nonmetastatic, since in mice of this strain such pulmonary tumors develop spontaneously. Many etiologic factors were given serious consideration by these investigators, but no positive conclusion was reached. The condition, it is pointed out, bears some analogy to a rare gastric adenopapillomatosis occurring in man and apparently having a familial tendency.

In marked contrast to the rarity of gastric cancer in animals is the well known frequency of carcinoma of the stomach in man. Livingston and Pack<sup>6</sup> estimated that 40,000 to 50,000 deaths are caused annually in the United States by gastric carcinoma. This is from 25 to 30 per cent of deaths due to malignant tumors, or about 3 per cent of all deaths. The mortality statistics of some European countries show that more than one half of all malignant neoplasms are carcinoma of the stomach (Konjetzny<sup>7</sup>).

In attempting to explain the marked difference between man and the lower animals in the development of spontaneous gastric cancer, attention naturally centered on the diet and on the fact that man is the only animal to heat his food and to consume it hot. In 1916 Lerche<sup>8</sup> analyzed the situation somewhat as follows. He reviewed statistics of the sites of carcinoma in the esophagus and stomach and the sites of cicatricial strictures resulting from swallowing corrosive fluids. The predisposition of certain sites to stricture formation following the ingestion of corrosive fluids was found to depend on the time of contact of the fluid with the various portions of the esophagus and stomach.

5 (a) Stewart, H. L., and Andervont, H. B. *Arch. Path.* **26** 1009, 1938.

(b) Stewart, H. L. *ibid.* **29** 153, 1940.

6 Livingston, E. M., and Pack, G. T. *End Results in the Treatment of Gastric Carcinoma*, New York, Paul B. Hoeber, Inc., 1939.

7 Konjetzny, G. E. *Der Magenkrebs*, Stuttgart, Ferdinand Enke, 1938.

8 Lerche, W. *Surg., Gynec. & Obst.* **23** 42, 1916.

Evidence was presented to show why cicatricial strictures do not occur at the sites of the physiologic narrowings but above them and why in cases in which a small amount of corrosive fluid was taken the esophagus escaped stricture formation entirely and only the antral area of the stomach was involved. The latter involvement was more frequent in nonfatal cases. It is significant that the favorite sites of carcinoma of the esophagus and stomach were found to correspond exactly to the favorite sites of cicatricial strictures in these organs. Since the explanation of the localization of corrosive action to certain areas depended on the mechanics involved when a fluid is ingested, it seemed reasonable to suspect some fluid used by man and not by animals as an etiologic factor in the production of carcinoma of the esophagus and stomach. Alcohol and other fluids were considered, but hot liquids remained as the most likely possibility.

Bonne<sup>9c</sup> had the unique opportunity to study two groups living in the same country with a marked difference in the incidence of gastric carcinoma—the Malays in Java, in whom gastric carcinoma is almost unknown, and the immigrated Chinese in Java, in whom it is not uncommon. In 3,885 autopsies on these Malays over a period of fifteen years about three fourths of whom were males, only 1 gastric carcinoma was found, although the total cancer rate when recalculated for a population of standard age was found to be in accord with the usual figures for Western countries. Bonne and co-workers<sup>9d</sup> found no significant difference in the morphologic character of the stomach or in the gastric secretion in these two groups. They carefully studied the dietary and other habits but were unable to draw definite conclusions. Somewhat similar studies have been reported repeatedly, for example, those of Herbert and Bruske<sup>10</sup> and Lintott,<sup>11</sup> who in attempting to account for the fact that the incidence of gastric carcinoma in Holland was twice that in England, concluded that an extrinsic factor was involved.

#### EXPERIMENTAL GASTRIC CARCINOMA

The animals almost universally used in reported investigations of experimental gastric carcinoma are mice and rats. Both the mouse and the rat have a stomach consisting of two chambers: (1) a forestomach comprising two thirds of the entire stomach and lined by squamous epithelium continuous with the esophagus and (2) a glandular stomach

9 (a) Bonne, C. *J Trop Med* **29** 288, 1926, (b) *Ztschr f Krebsforsch* **25** 1, 1927, (c) *Am J Cancer* **30** 435, 1937 (d) Bonne, C., Hartz, H., Klerks, J. V., Posthuma, J. H., Radsma, W., and Tjokronegoro, S. *ibid* **33** 265, 1938 (e) Bonne, C., and Sandground, J. H. *ibid* **37** 173, 1939

10 Herbert, W. E., and Bruske, J. S. *Guy's Hosp Rep* **86** 301, 1936

11 Lintott, G. A. M. *Guy's Hosp Rep* **86** 293, 1936

separated from the upper squamous-lined portion by a slight elevation, the limiting ridge

Obviously two entirely different types of carcinoma might develop (1) a squamous cell carcinoma of the nonsecreting forestomach, which would appear to be more closely related to a carcinoma of the esophagus or skin than to (2) an adenocarcinoma of the secreting glandular stomach. A sharp distinction between these two unrelated types should be made.

The means used in attempts to produce experimental gastric carcinoma generally fall into one of the following categories: (1) dietetic irritants, (2) parasites, (3) dietary deficiencies, and (4) agents known or suspected to be carcinogenic when fed, injected or implanted.

*Dietetic Irritants*—The only attempt to produce gastric cancer experimentally by hot food was made by Lewis<sup>12</sup> a good many years ago. He poured hot mush daily into the gastrotomies of 35 dogs. Unfortunately, none of the dogs survived this procedure more than one year. No evidence of gastric carcinoma, gastric ulcer or gastritis was found. If this procedure could lead to changes eventually resulting in gastric carcinoma, it is now known, as pointed out by Wells, Slye and Holmes,<sup>3</sup> that it would probably require a longer time than these dogs survived. Experimental cancers of any kind are rare in dogs, but Passey's<sup>13a</sup> recent observation of a malignant melanoma occurring in a dog after six and one-half years of weekly tarring may give some indication of the induction period required.

*Parasites*—The earliest attempts at experimental production of cancer to attract wide attention were those of Fibiger, who in 1913 published the first of a series of beautifully illustrated reports on the production of squamous cell carcinoma in the forestomachs of rats by means of a nematode, *Gongylonema neoplasticum*.<sup>14</sup> Much of this work has been criticized and remains unconfirmed (Passey, Leese and Knox<sup>13b,c</sup>, Cramer<sup>15</sup>).

Fibiger found squamous cell tumors in the forestomachs of wild rats infected with *Gongylonema neoplasticum* (*Spinoptera neoplastica*). Rats contracted the infection from cockroaches. Fibiger fed infected cockroaches to black and white laboratory rats and maintained them on a diet of white bread and water. After infection none of the rats lived more than two hundred and ninety-eight days. The changes in the stomach were limited to the squamous-lined forestomach. The glandular portion was never involved either primarily or by invasion. The stomach was often

<sup>12</sup> Lewis, J. H., cited by Wells, Slye and Holmes<sup>3</sup>

<sup>13</sup> (a) Passey, R. D. *J. Path. & Bact.* **47** 349, 1938. (b) Passey, R. D., Leese, A., and Knox, J. C. *ibid.* **40** 198, 1935, (c) **42** 425, 1936.

<sup>14</sup> Fibiger, J. *Ztschr. f. Krebsforsch.* (a) **13** 217, 1913, (b) **14** 295, 1914, (c) *J. Cancer Research* **4** 367, 1919.

<sup>15</sup> Cramer, W. *Am. J. Cancer* **31** 537, 1937.



enlarged, with nodular prominences on the external surface. The gastric mucosa had obvious papillary growths, and metastases were reported in the lungs of some rats. Microscopically, simple hyperplasia, hyperkeratosis and slight papillary downgrowths of the squamous epithelium were found in almost all rats. These changes were regarded as benign. In addition, over 50 per cent of the rats had changes interpreted as carcinomatous. In the first 111 rats reported on, Fibiger found, according to his criteria, 19 cases of squamous cell carcinoma, and later he observed similar cases in other rats. The changes in the forestomach were classified as malignant when the following conditions were present: (1) downgrowth of atypical and keratinized epithelial cells in abundance, arranged as spherical masses and horny globes, (2) infiltrative growths of these into the deeper layers, splitting up invasively the connective tissue of the mucosa and the muscle cells of the muscularis mucosae and also penetrating into the superficial and deeper layers of the submucosa. Neither infiltration of the muscularis or neighboring structures nor metastases were required for the designation of the tumors as malignant. As a matter of fact, the muscularis was not invaded even in those cases in which metastases were reported. Whether these represented actual metastases to the abdominal lymph nodes and the lungs is open to serious doubt. Lymph nodes similar to those believed by Fibiger to have metastatic deposits have been observed in the absence of malignant growth. Lesions in the lungs similar to those regarded as metastases have been seen repeatedly in rats without gastric lesions. They are usually bronchiectatic cavities or metaplastic epithelium.<sup>13c</sup>

As Cramer<sup>15</sup> recently pointed out in an excellent review of the subject, Fibiger's results have rarely been questioned, although many investigators seriously doubt his interpretation of the lesions as malignant. In the final analysis, much depends on whether metastases took place. Fibiger pointed out that the metastases to the lymph nodes were only microscopic, but the microscopic appearance of the so-called metastases is not conclusive.

Fibiger<sup>14c</sup> later reported a squamous cell carcinoma in the forestomach of a mouse 20 to 22 months old which died four hundred and twenty-eight days after *Spiroptera* infection. The tumor involved all layers of the stomach and invaded the liver, spleen and diaphragm. Numerous metastases were found in the abdomen. The metastases were successfully transplanted through four generations covering a period of one year, in the cases of 28 of the 55 mice that survived inoculation, and maintained the histologic features of the original gastric tumor. Fibiger regarded this as the same type of lesion he had observed in rats and emphasized the fact that this mouse lived more than four months longer, after *Spiroptera* infection, than any rat and that therefore the advanced characteristics of a malignant tumor

had time to develop Passey, Leese and Knox,<sup>13b</sup> however, succeeded in keeping rats on a complete diet alive for four hundred and eighty days after infection with *Gongylonema neoplasticum* and observed only slight mucosal changes in the forestomach. This suggests that the *Spinoptera* infection in itself is not sufficient to cause the gastric lesions. There is little doubt that the gastric tumor in the mouse reported by Fibiger was a squamous cell carcinoma, for the muscularis and neighboring organs were invaded, and transplantation of the metastases was successful. Fibiger never reported a similar tumor in a rat, although a few similar but not as well established ones were found in mice.

Passey, Leese and Knox<sup>13b</sup> recently repeated Fibiger's experiments. As already mentioned, in a group of rats on a complete diet infected with *Gongylonema neoplasticum* the mucosal changes in the forestomach were insignificant. In a group of rats fed Fibiger's diet of white bread and water some lesions occurred in the forestomach, although they were not as severe as those seen in a similar group infected with *Gongylonema neoplasticum*. No gastric carcinoma, however, was found in any rat. Similar results were obtained in rats on a diet deficient in vitamin A with added parasitic infection. These investigators pointed out that Brumpt had observed only 1 gastric carcinoma in 781 wild rats found infected with *Gongylonema neoplasticum* and had failed to obtain a single carcinoma in 31 laboratory rats and 5 white mice experimentally infected with the parasite.

Bullock and Rohdenburg<sup>16</sup> believed that the lesions of the forestomach observed by Fibiger were benign and the result of nonspecific chronic irritation. They devised many ingenious experiments to test this hypothesis. They were unable to duplicate Fibiger's results exactly but produced a close resemblance. Three types of gastric irritation were used: (1) mechanical, (2) chemical and (3) a combination of the two.

A celluloid ball covered with pig's bristles was introduced into the rat's forestomach through a gastrotomy and suspended by a string. Within a few weeks irregular polypoid growths were observed, most marked at the site of incision. In control experiments, however, in which a simple purse string suture was taken in the stomach wall, without making an incision, the same changes were sometimes noted. In addition such polypoid growths were seen in some rats that were not operated on. When a cork ball with protruding pinpoints, covered with celluloid and keratin, was introduced into the stomach through a gastrotomy and allowed to remain free in the gastric cavity, lesions were usually found in the glandular chamber of the stomach and were more severe when the gastrotomy had been in the glandular portion. The

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<sup>16</sup> Bullock, F. D., and Rohdenburg, G. L. *J. Cancer Research* **3**: 227, 1918.

changes consisted of an increase in the mucous cells and a complete absence or scarcity of the parietal cells with downgrowths of glandular epithelium forming cystadenomas and accompanied by varying degrees of inflammation of the gastric wall. It is interesting that in some cases osteoid tissue was found in the connective tissue of the stratum proprium. When a rubber sponge impregnated with scarlet red powder or pine tar was introduced into the stomach, hypertrophy and proliferation of the squamous epithelium, especially around the gastrotomy, took place and sometimes penetrated the muscularis mucosae. Similar changes were found after scarlet red or pine tar in oil or ether had been injected directly into the stomach wall. Although the factor of a food deficiency may have played some role in these results, Bullock and Rohdenburg clearly showed that lesions of the forestomach resembling those observed by Fibiger could be produced in the absence of parasites. Furthermore, the well illustrated benign cystadenomatous lesions in the glandular stomach form an instructive basis of comparison with similar lesions which have at times been reported as malignant.

Yokogawa<sup>17</sup> reported the experimental production of squamous cell carcinoma in the forestomach of the rat by means of another nematode, *Gongylonema orientale*. Carcinoma was reported to have developed in 3 of 61 white rats after at least two hundred days of infection. The characteristics of these tumors were in all essential respects similar to those observed by Fibiger. The tumors were papillomatous growths which at their base extended down through the muscularis mucosae into the submucosa but did not involve the muscularis. Invasion of lymphatic spaces was present. The glandular portion of the stomach showed some atrophic change but was otherwise not involved. Metastases to the liver and lung were reported in one of the rats. Yokogawa described these metastases grossly as hard grayish spots containing a cheesy material. Microscopically, the cavities were lined in part by laminated flat cells. Yokogawa recognized that such lesions were sometimes found in rats without cancer but thought the evidence in this particular case pointed to metastases. A photomicrograph of one such lesion leaves some doubt whether this was actually a metastasis. Yokogawa's observations are essentially similar to those of Fibiger, and their interpretation is open to the same objections.

Bonne<sup>9a</sup> reported a squamous cell tumor in the forestomach of a wild rat caught in Italy and found infected with a nematode, *Hepaticola gastrica*. The tumor was a papilloma the size of a cherry. Microscopically, the muscularis mucosae was penetrated by an epithelial downgrowth, but the muscularis was uninvolved. The glandular stomach was normal. No metastasis was found. Evidence that this was

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17 Yokogawa, S. Gann **18** 48, 1925

a malignant growth rests chiefly on the fact that the tumor invaded some blood vessels. Bonne pointed out the close resemblance to Fibiger's tumors and the probability that the nematode infection of the stomach was casual rather than coincidental. Whether this represented a true cancer of the forestomach can be questioned.

Vogel<sup>18</sup> later experimentally inoculated rats with *Hepaticola gastrica*. Although he failed to obtain infection of old rats, he succeeded in producing infections in 4 young ones, in 2 of which squamous cell carcinoma of the forestomach was reported to have developed after three months. Vogel noted that there was considerable similarity to the lesions observed by Fibiger with the exception of a greater tendency toward epithelial downgrowth into the submucosa and a lesser amount of papillomatosis into the lumen. The muscularis was not involved, and the glandular stomach was normal. Metastases are not mentioned. Vogel stressed the fact that no parasites were found in the tumors when the animals died and regarded this as evidence that a true malignant growth was present in that after the malignant change had taken place it persisted without the presence of the casual agent. Although this is one explanation it is not by any means the only one.

Bonne and Sandground<sup>9c</sup> recently noted the association of a "gastric adenoma bordering on malignancy" and a nematode, *Nochtia nochtii*, in Javanese monkeys. The adenoma was never observed in the absence of the parasite, and whenever the parasite was found, an adenoma was present. These authors succeeded in inoculating monkeys with *Nochtia nochtii* and producing such tumors experimentally. No metastases were observed, but definite invasion of blood vessels was noted. Whether these adenomas can develop into adenocarcinomas remains an open question.

Comment. A review of gastric parasitic infections does not disclose conclusive evidence that infection in itself leads to gastric tumors in spontaneously or experimentally infected rats. The tumors observed in such animals may be due primarily to other factors not satisfactorily eliminated in the experiments, such as dietary deficiencies. When Passey, Lesse and Knox<sup>13b</sup> inoculated rats maintained on a complete diet with *Spinoptera neoplastica*, insignificant gastric mucosal changes resulted. It is therefore not surprising that some investigators found more severe gastric lesions in young and growing rats than in old ones. In the interpretation of reports, due allowance should be made for the variation of different species and strains of animals in their reaction to the same dietary deficiency or parasite. The role of nematode infections in the genesis of forestomach lesions in rats seems to be that of aggravating mucosal changes initiated by other causes, although parasites,

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<sup>18</sup> Vogel, H. Ztschr. f. Krebsforsch. **29** 351, 1929.

as is well known, can cause tissue proliferation and malignant growth in other organs. Hoepple,<sup>19</sup> in a comprehensive review of parasites and tumor growth, discussed the various mechanisms by which this may occur. Bonne and Sandground's<sup>20</sup> observations indicate, however, that in monkeys inoculated with *Nochtia nochtii* gastric adenomas occur only in the presence of parasitic infection.

Regardless of the exact cause of the gastric squamous cell tumors attributed to parasites, it is of interest to consider the evidence of the malignant nature of these lesions. Fibiger's<sup>11c</sup> clearly stated criteria of malignancy, previously mentioned, will be more fully considered later. It is, however, generally accepted at present that his criteria are inadequate. Whether such changes can be regarded as "precancerous" will remain an open question until it is possible to observe more frequent transition to undoubted malignancy. Fibiger reported squamous cell tumors in a few mice, one of which was supported by practically conclusive evidence of malignancy, however, in view of the large number of animals used in Fibiger's work the possibility of spontaneous malignancy was not entirely eliminated. The causal relation between gastric squamous cell carcinoma, parasites and other factors that may have been unknowingly involved needs to be demonstrated more regularly to be convincing.

*Dietary Deficiencies*—Dietary deficiencies may seem unrelated to experimental gastric carcinoma, but the severe changes in the forestomach apparently caused by such deficiencies, particularly in rats, have been reported at times as malignant. Furthermore, because these severe changes closely simulate those sometimes interpreted as carcinoma and attributed to other causes, a food deficiency should be considered as a possible factor in some of the reports of experimental gastric carcinoma.

Pappenheimer and Larimore,<sup>20a</sup> in 1923, were the first to call attention to the possible relation of forestomach lesions in rats to a dietary deficiency. Their preliminary evidence suggested that vitamin A deficiency might be the cause. Further study,<sup>20b</sup> however, revealed that the lesions were present when sufficient ultraviolet radiation was given to prevent rickets. The lesions were uncommon in rats on a complete diet, and when present, were mild. When rats were placed on a diet known to bring about the forestomach lesions, neither the addition of cod liver oil or of Osborne and Wakeman's yeast extract or changes in the mineral content of the diet prevented them. Pappenheimer and Larimore concluded that a food deficiency was a factor in the appearance

19 Hoepple, R. Chinese M. J. **47** 1075, 1933.

20 Pappenheimer, A. M., and Larimore, L. D. (a) Proc. Soc. Exper. Biol. & Med. **21** 141, 1923-1924, (b) J. Exper. Med. **40** 719 1924.

of the gastric lesions but were unable to determine what element of diet was lacking. They observed that rats on a deficient diet often had hair in their stomachs and suggested that this hair might aggravate the lesions. Of 6 rats fed a complete diet mixed with ground-up hair, 2 were found to have benign changes of the forestomach and ulcers, with hair embedded in the stomach in such positions as to indicate that it was a factor in the production of the ulcers. The lesions observed by Pappenheimer and Larimore were limited to the forestomach, usually to an area near the elevated ridge separating the forestomach from the glandular stomach, and consisted essentially of papillomatosis. The changes were considered benign.

Wolbach and Howe<sup>21</sup> in 1925 reported change caused by vitamin A deficiency in rats. They took precautions to prevent any deficiency other than vitamin A by forced feeding whenever necessary, particularly after anorexia and impairment of the sense of smell developed. No significant gastric lesion was observed in spite of extensive changes in other covering epitheliums. Wolbach and Howe found large bronchiectatic cavities in many of the rats, which were often considered the immediate cause of death. Although tissue changes in the stomach were not found, the alteration in the covering epithelium caused by vitamin A deficiency is worth noting. "Growth activity of the epithelium is not diminished, on the contrary, there is convincing evidence that it is greatly augmented. In a few of our animals, the behavior of the replacing epithelium in respect to numbers of mitotic figures and response on the part of connective tissue and blood vessels suggests the acquisition of neoplastic properties." Because of the thoroughness of this work, the observations in relation to the stomach and lungs seem particularly significant. It has been suggested that the so-called metastases to the lungs in rats with gastric tumors were sometimes bronchiectatic cavities. That such cavities do appear in rats without any pathologic changes in the stomach was clearly shown by Wolbach and Howe and has been repeatedly confirmed by others.

Fujimaki<sup>22a, b</sup> in 1926 observed severe papillomatosis of the forestomach in young rats on a vitamin A-deficient diet. The lesions were believed to be malignant. One of 5 rats with the most severe gastric papillomatosis was reported to have metastatic nodules in the lung. One of these metastases is illustrated<sup>22b</sup>. The gastric lesions consisted essentially of hyperkeratosis and downgrowth of atypical epithelium with penetration of the muscularis mucosae. The muscularis was not invaded. The glandular stomach was normal. An accompanying editorial note

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21 Wolbach, S. B., and Howe, P. R. *J. Exper. Med.* **42** 753, 1925.

22 (a) Fujimaki, Y. *J. Cancer Research* **10** 469, 1926, (b) *Gann* **21** 8, 1927. (c) Fujimaki, Y., Arimoto, K., Kimura, T., Ohba, K., and Matsuda, G. *Tr. Jap. Path. Soc.* **21** 708, 1931.

states that microscopic examination of Fujimaki's slides, from which the photomicrographs were prepared, left some doubt as to the malignant nature of the changes <sup>22a</sup>. In a more recent publication Fujimaki and co-workers <sup>22c</sup> offered evidence to show that various fatty acids and lipoids in the diet are factors in the appearance of atypical epithelial proliferation of the forestomach in rats and that vitamin A (butter) does not prevent it.

Cramer's <sup>15</sup> experiments with vitamin A-deficient rats are worthy of note for two reasons. First, the severe papillomatosis of the forestomach was considered benign, and, second, two experiments done ten years apart, with apparently identical methods except that different strains of rats were used, showed a startling difference in results. Cramer stated that if the earlier sections of the stomach had not been available for reexamination he himself could hardly have believed that the benign papillomatosis, as marked as that observed by Fibiger, could have been so severe in the first experiment and so mild in the second. He has no explanation to offer except that different strains of rats react in varying degrees to vitamin A deficiency or that the papillomatosis of the forestomach may be due to a virus the action of which is enhanced in rats on a deficient diet.

Hoelzel and Da Costa <sup>23</sup> offered a hypothesis of the genesis of the forestomach lesions in rats observed by them without postulating any specific food deficiency. They found that rats on a low protein diet when fed alternately for two days and starved for two days had ulcers of the forestomach after two weeks. These authors stressed the presence of the ulcers, although some papillomatosis apparently also was present. The simplest method of producing such lesions was found to be starvation. Inanition alone, however, did not cause the ulcers to persist, as shown by the fact that they healed readily on a bran diet which had no caloric value. Ulcers also developed in rats on a bread diet. The incidence of the ulcers decreased when 15 to 30 per cent calcium carbonate was added to the bread. Ingestion of hain seemed to cause ulceration only when this interfered with a normal intake of food. Their observations led them to conclude that the acid-combining properties of the food largely determined the amount of ulceration and that the acid gastric juice probably acted as an irritant in the manner shown by Bullock and Rohdenburg <sup>16</sup> by artificial irritation.

Rats fed a high fat, low protein diet by Hoelzel and Da Costa <sup>23b</sup> were found to have a particularly prominent overgrowth of the mucosa of the forestomach. This observation is similar to that of Fujimaki and co-workers, <sup>22c</sup> who found that an increase in papillomatosis resulted in

<sup>23</sup> Hoelzel, F, and Da Costa, E. Proc Soc Exper Biol & Med (a) **29** 382, 1931, (b) **29** 385, 1932

rats fed various fatty acids and lipoids. Because these lesions resembled those previously reported by some investigators as malignant, Hoelzel and Da Costa made a study of the morphologic changes and found that grossly they consisted of nodular prominences on the outer surface of the stomach with ulceration and overgrowth of the mucosa. Microscopically, the largest nodules were found to be epithelial cysts, generally located at the limiting ridge. Other nodules consisted of cornified globular masses. In most cases continuity with the normal epithelial layer could be demonstrated. Although there was a downward growth of the epithelium, the muscularis was never invaded. Observations on 7 rats which were fed alternately in a manner that produced ulcers and nodules in control rats and which then were given a complete diet indicated that these rather severe changes were reversible. The ulcers disappeared faster than the nodules or epithelial cysts, but even these seemed to disappear eventually. Hoelzel and Da Costa concluded that the lesions were not malignant. It is unknown whether they would have become malignant in a more prolonged experiment.

Harde<sup>24</sup> reported a squamous cell carcinoma in the forestomach of a mouse fed a deficient diet for seven months. Because of a paratyphoid infection at the end of this time, a complete diet was given and continued until the mouse died seven months later. A squamous cell tumor, 1 by 2 cm., was found in the forestomach, which infiltrated all layers of the gastric wall and invaded the liver. In addition there was adenomatosis of the glandular stomach, which infiltrated the submucosa. Whether metastases were present is not known. There is no illustration. Harde made no attempt to correlate the experimental procedure with the appearance of the carcinoma since this was the only cancer in over 600 mice.

Findlay<sup>25</sup> in 1928 reported lesions in rats due to vitamin B<sub>1</sub> and vitamin B<sub>2</sub> deficiency. He found papillomatosis, consisting of thick layers of squamous cells with exfoliation of the keratin layer, in the forestomach of every rat fed a diet deficient in vitamin B<sub>2</sub>. The epithelium formed papillary projections into the lumen, and blunter processes extended downward as far as the muscularis mucosae. There were numerous mitotic figures. Ulceration of the superficial layers of the mucosa was present. No evidence of malignancy was found. Such gastric changes were never present in rats on vitamin B<sub>1</sub> deficiency only. Findlay agreed with Pappenheimer and Laimore that the ingestion of hair might afford a plausible explanation of the lesions, since vitamin B<sub>2</sub>-deficient rats shed large amounts, which inevitably contaminated the diet. Among other lesions found in vitamin B<sub>2</sub>-deficient rats Findlay

24 Harde, E. *Compt rend Soc de biol* **110** 245, 1932

25 Findlay, G. M. *J Path & Bact* **31** 353, 1928



reported profound changes in the squamous epithelium of the skin, consisting of active mitosis in the cells of the stratum granulosum, while the whole process of keratinization was excessively active. All the changes are well illustrated.

Sharpless<sup>26a</sup> observed lesions developing in the forestomachs of rats within three months after they were placed on a low casein diet. These lesions were described essentially as thickening of the epithelial ridges with papillomatosis, hyperkeratosis and ulceration of the mucosa, most marked along the limiting ridge. No metastases were observed.

In a later report Sharpless<sup>26b</sup> described hyperplastic changes of the forestomach in all of the 125 rats fed a low casein diet and no such lesion in 200 control rats on a complete diet. This stock of rats has shown no spontaneous malignant tumor in over four years. Control rats fed the same diet with the addition of 12 per cent vitamin-free casein or 0.2 per cent cystine showed no lesions. When given a low casein diet with the addition of 10 per cent gelatin, to approximate the physical properties of a diet without a protein deficiency, the lesions appeared as though a low casein diet only had been fed. The papillomatosis in some instances almost occluded the lumen. In cases of long standing, spurs of atypical, keratinizing epithelium sometimes penetrated the muscularis mucosae extending into the submucosa, and proliferated, forming cysts. Active invasion of the muscularis was reported in some rats. As many as eighteen mitotic figures were observed in one high power field. No metastases were found. One of several photomicrographs has a legend suggesting a malignant process. Sharpless regarded some of these lesions as malignant, however, the evidence has been considered inadequate.<sup>26c</sup>

Howes and Vivier<sup>27</sup> reviewed the relation of diet to the occurrence of gastric lesions in rats and the interpretation of these changes. In their own work they found that whole yeast prevented the forestomach lesions when added to the diet used by Pappenheimer and Lammore to produce them, whereas the addition of Osborne and Wakeman's yeast extract, vitamin-free casein, cod liver oil or other forms of vitamin A was of no protective value. The gastric changes observed when whole yeast was not added to the diet were essentially hyperplasia, hyperkeratinization and ulceration of the squamous epithelium in the forestomach, with hypertrophy of the limiting ridge. Similar lesions without keratinization were found in the glandular stomach. Mitotic figures were numerous. The muscularis mucosae was thinned but intact. Hair was found in the stomachs of only 2 rats and therefore was not regarded

26 Sharpless, G. R. (a) *Proc. Soc. Exper. Biol. & Med.* **34** 684, 1936, (b) *Ann. Surg.* **106** 562, 1937, (c) abstracted, *Am. J. Cancer* **37** 457, 1939.

27 Howes, E. L., and Vivier, P. J. *Am. J. Path.* **12** 689, 1936.

as the primary cause of the lesions Pappenheimer and Laimone's failure to prevent the lesions with Osborne and Wakeman's yeast extract was regarded as due to the lack of something which is present in whole yeast Their explanation of the apparently conflicting views of the role of vitamin A deficiency was that Wolbach and Howe had previously observed no gastric lesions in rats on a diet deficient only in vitamin A Since anorexia is a prominent symptom in vitamin A deficiency, secondary deficiencies are likely to develop Howes and Vivier believed these secondary deficiencies to be a factor in the appearance of the lesions

Comment A review of the gastric lesions present in animals on various deficient diets, particularly those in the forestomachs of rats, does not disclose sufficient evidence to warrant the conclusion that they are definitely malignant There is a striking similarity between the forestomach changes in rats on deficient diets and those observed in rats with nematode infections This resemblance is so close that it has been suggested a dietary deficiency is the primary cause of the parasitic tumors Whether a specific deficiency is responsible for the lesions is still a controversial matter Cramer<sup>15</sup> suggested a possible mechanism by which dietary deficiencies may lead to papillomatosis of the forestomach He reviewed the conflicting evidence of its cause appearing in the literature and the highly inconsistent results in his own experiments, previously mentioned Although different strains of animals vary in their reaction to the same deficiency, this may not adequately account for all observations The fact that some cutaneous papillomas can be transmitted by means of a virus led Cramer to speculate that the primary cause of papillomatosis of the forestomach may possibly be a virus whose action is enhanced in rats fed a deficient diet This hypothesis in one form or another has been previously suggested by others, but Cramer's experiments are an example of its possibility and might explain, among other things, the suspected occasional endemic occurrence of forestomach papillomatosis in rats

Lesions of the glandular stomach have been observed in rats on deficient diets<sup>27</sup> but much less commonly than those of the forestomach, and they have not been regarded as malignant

A question of prime importance in relation to gastric lesions resulting from dietary deficiencies of one sort or another is that of the reversibility of the changes This possibility has not been satisfactorily eliminated in the more severe lesions sometimes regarded as malignant It seems essential to determine whether the gastric changes will persist and progress invasively after the resumption of a complete diet The evidence presented by Hoelzel and Da Costa<sup>23b</sup> indicates that the lesions they observed, possibly not as severe but apparently of the same type as those sometimes simulating malignant growth, are reversible

*Carcinogenic Agents*—1 Tar With the advent of tar as a carcinogenic agent, attention was directed primarily to experimental tumors in organs or tissues other than the stomach, although some attempts to produce gastric carcinoma with tar have been reported. Ishibashi and Ohtani<sup>28</sup> found definite adenomas, not regarded as malignant, fifty days after the injection of tar into the submucosa of the stomach in rabbits.

Buschke and Langer<sup>29</sup> noted forestomach lesions in 50 of 54 rats after rectal injections of tar once to twice a week for four to six months. The animals were 3 to 5 months old at the beginning of the experiment. The first rectal injection led to rather severe general reactions although subsequent injections were well tolerated for the next two to three months, and then there was a progressive decline in general health. The animals were allowed to die spontaneously. The lesions were limited to the forestomach and were usually near the ridge separating it from the glandular chamber. The rectum, glandular stomach and intestine showed no significant changes. An excellent description and photographs of the lesions bear out the close resemblance, as pointed out by the authors, to those noted by Fibiger. The changes consisted essentially of ulceration, hyperkeratosis and papillomatosis of the mucosa with a downgrowth of the epithelium, sometimes extending through the muscularis mucosae into the submucosa and part of the muscularis but never through the serosa. Mitotic figures were common. The careful pathologic analysis of the lesions is noteworthy. Although some regional lymph nodes were enlarged, no metastatic tissue was found microscopically nor were metastases found elsewhere. Nests of epithelium sometimes appeared to be isolated, but a connection with the surface epithelium was found. This was not always obvious, and the authors pointed out that the connection might easily have been overlooked in some sections. In addition, the epithelial downgrowths did not seem to infiltrate the deeper structures but rather to push through them, even to the extent of giving the external surface of the stomach a nodular appearance. In view of these observations the lesions were not considered malignant, although the possibility that in time a malignant change might have occurred was discussed. The relation of the rectal injections of tar to the lesions of the forestomach is of considerable interest. Buschke and Langer considered but dismissed the possibility that some tar was swallowed. No gastric parasites were found. They felt the changes were due to the general effect of absorbed tar. In retrospect it may be questioned whether the tar had any specific effect. The animals were allowed to die spontaneously and were reported to have been in a poor nutritional state for some time before death. Pre-

28 Ishibashi, M., and Ohtani, S. *Gann* **15** 2, 1921.

29 Buschke, A., and Langer, E. *Ztschr. f. Krebsforsch.* **21** 1, 1923.

sumably there was anorexia with consequent inadequate intake of food and dietary deficiencies such as have been found to lead to similar alterations in the stomach

Bonne,<sup>9b</sup> in an excellent paper dealing in part with gastric lesions in tarred animals, found benign papillomas in the forestomachs of a few mice. Bonne thought these were probably caused by swallowed tar. When tar was applied to the mouths of 50 mice, the incidence of papillomas definitely increased, and 1 mouse was reported to have had a squamous cell carcinoma of the forestomach. Tar had been applied orally twice a week for thirty-three weeks and then discontinued. At the time of death, one year after tarring was started, this mouse had a carcinoma of the lower lip and buccal mucosa in addition to the gastric tumor, which penetrated the muscularis mucosae and infiltrated almost to the serosa. No metastasis was found. No further description or illustration of this tumor is given. No malignant gastric lesion was found in 20 rats repeatedly tarred by mouth. A papilloma in the forestomach of 1 rat and some benign changes in the glandular stomach of another were regarded as spontaneous, since similar lesions had been observed in untarred rats. A third rat tarred by mouth twice a week for twenty weeks was found to have bronchopneumonia and two grossly visible white nodules in the lung. Microscopic examination disclosed a large keratinized mass in the periphery of the lung. Squamous epithelium was recognizable at the borders of this mass, which was nowhere seen to be related to a bronchus. The remainder of the lung could not be examined for further squamous cell metaplasia. This finding is interesting, since no squamous cell tumor was found elsewhere to suggest a metastatic lesion in the lung. A photomicrograph beautifully illustrates the keratinized pulmonary mass.

Voronoff and Alexandrescu<sup>30</sup> fed 10 white rats a mixture of tar, hydrous wool fat, aniline oil and toluylenediamine three to four times a week. A peritoneal sarcoma and an adenocarcinoma of the prepyloric portion of the stomach were reported in a rat dying six months later. The latter tumor is described as infiltrating all layers of the stomach, with metastases to the liver and retropyloric lymph nodes. Drawings of the microscopic appearance of this lesion show the superficial gastric mucosa, not including the muscularis mucosae nor the deeper layers of the stomach. Some mucosal ulceration and various alterations of the glandular structure are evident, but the drawings are not clear enough to permit an objective interpretation. There is no illustration of a metastasis. This work apparently has not been repeated by the same or other investigators.

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30 Voronoff, S, and Alexandrescu, G. *Néoplasmes* 8 129, 1929

Tani<sup>31</sup> noted marked papillomatosis of the forestomach in a few rats given tar orally. The changes were severe enough to suggest a malignant process. Injections of tar into the gastric wall were found to be less effective. Microscopic studies were not found in the reports available.

Twort and Twort<sup>32a</sup> in a detailed report of lesions found in 60,000 tarred mice, state that while they were not primarily interested in the alimentary tracts of their animals and hence may have overlooked some gastric lesions, they noted occasional papillomas of the forestomach, more frequently in those mice painted with tar for many weeks than in those painted for only a short time. One mouse had a pronounced benign adenomatous or hyperplastic condition of the glandular portion of the stomach. It was, of course, impossible to say whether 1 glandular lesion in 60,000 mice was casually related to the tarring or to the swallowed tar. They found no gastric carcinoma in any of the 60,000 mice.

Mercier and Gosselin<sup>33</sup> injected coal tar in olive oil intraperitoneally into a mouse, which died four months later. A tumor the size of a large pea was found in the forestomach near the limiting ridge. It is briefly described as infiltrating the musculature. The tumor was considered malignant and was regarded as the result of irritation by swallowed hair contaminated with tar rather than of the intraperitoneal injection of coal tar in oil. It is not clear whether metastases were present. There is no illustration.

Reinhard and Candee<sup>33a</sup> fed mice 10 mg. of coal tar in butter once a week for seven months and observed no gastric tumors.

2. Carcinogenic Chemicals. With the isolation and synthesis of a carcinogenic constituent of tar and the preparation of many other carcinogenic agents, it became possible to feed animals single chemical compounds of known carcinogenic potency for some tissues and organs. In the meantime the carcinogenic properties of some other substances were recognized or suspected. Some of these have been used in attempts to induce gastric carcinoma in animals, usually by feeding, although other methods have been employed as well.

(a) Diaminoazobenzene. Otsuka<sup>34</sup> in 1935 noted papillomatosis of the forestomach in mice fed diaminoazobenzene in olive oil daily. A closely related compound, dimethylaminoazobenzene, has been used in

31 Tani, I. *Tr. Jap. Path. Soc.* **21** 715, 1931.

32 (a) Twort, J. M., and Twort, C. C. *J. Path. & Bact.* **35** 219, 1932.

(b) Twort, C. C., and Bottomley, A. C. *Lancet* **2** 776, 1932.

33 Mercier, L., and Gosselin, L. *Compt. rend. Soc. de biol.* **113** 669, 1933.

33a Reinhard, M. C., and Candee, C. F. *Am. J. Cancer* **26** 552, 1936.

34 Otsuka, I. *Gann* **29** 209, 1935.

the experimental production of carcinoma of the liver in rats but causes no malignant lesion in the stomach (Kinosita<sup>35</sup>) Diaminoazobenzene, on the other hand, has little effect on the liver Otsuka observed papillomatosis as early as fifty-nine days after diaminoazobenzene was fed and found that it was present in all mice surviving three hundred and seven days or more There was no invasion of the muscularis and no metastasis The changes were considered benign The glandular stomach was not involved

(b) 1,2,5,6-Dibenzanthracene Reports of gastric lesions following administration of 1,2,5,6-dibenzanthracene are uncommon

Perry and Leonard<sup>36</sup> reported that carcinoma of the stomach developed in some mice painted twice a week with 0.3 per cent 1,2,5,6-dibenzanthracene in benzene In mice painted with theelin in addition to dibenzanthracene more tumors developed, but it is not stated whether this included gastric tumors It is not clear how long the mice with gastric tumors had been painted There is no further description No mention is made of the type of neoplasm—squamous or glandular There is no illustration of a gastric tumor

Reinhard and Candee<sup>37</sup> fed mice 0.02 per cent dibenzanthracene in butter for seven months without obtaining gastric or other tumors

Ilfield,<sup>37</sup> using Shear's method of incorporating carcinogenic hydrocarbons in cholesterol pellets, implanted 5 per cent dibenzanthracene pellets in the wall of the stomach of a dog No tumor was found after one year Similar pellets placed under the gastric serosa of ferrets did not cause a tumor at the time of report, four months later

Cook and co-workers<sup>38</sup> reported that no carcinoma of the alimentary tract resulted from feeding dibenzanthracene in lard for an unspecified length of time to mice and rats

Branch<sup>39</sup> painted the skin of mice twice a week with 0.5 per cent dibenzanthracene in benzene and noted considerable licking of the painted areas with the resultant ingestion of dibenzanthracene, but no tumor of the gastrointestinal tract was found in any mouse

Van Prohaska, Brunschwig and Wilson<sup>40</sup> failed to obtain gastric lesions in white mice by feeding dibenzanthracene in lard three times a week for six months

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35 Kinosita, R. *Tr. Jap. Path. Soc.* **27** 665, 1937

36 Perry, I., and Leonard, G. L. *Am. J. Cancer* **29** 680, 1937

37 Ilfield, F. W. *Am. J. Cancer* **26** 743, 1936

38 Cook, J. W., Haslewood, G. A. D., Hewitt, C. L., Hieger, I., Kennaway, E. L., and Mayneord, W. V. *Am. J. Cancer* **29** 219, 1937

39 Branch, C. F. *Am. J. Cancer* **26** 110, 1936

40 Van Prohaska, J., Brunschwig, A., and Wilson, H. *Arch. Surg.* **38** 328, 1939

Fieser,<sup>41</sup> in a comprehensive review of the relative carcinogenic properties of various polynuclear aromatic hydrocarbons, found that 1,2,5,6-dibenzanthracene usually requires a longer time to induce skin and subcutaneous tumors in mice and rats than some of the others. The possibility that gastric lesions might result in animals fed dibenzanthracene for longer periods is therefore not entirely excluded.

(c) Cholesterol Compounds. Roffo<sup>42a</sup> reported the experimental production of adenocarcinoma of the stomach in rats. All rats were 3 months old at the beginning of the experiment and were maintained on a diet of bread and milk for sixteen to twenty-six months thereafter. A control group of rats fed this diet or bread, milk and unirradiated cholesterol showed no gastric lesions similar to those observed when various additions to the diet were made. The daily additions consisted of 100 mg of forty-eight hour ultraviolet-irradiated cholesterol, heated cholesterol, sun-treated or ultraviolet-irradiated egg yolk. One group was fed ultraviolet-irradiated bread and milk. In all, 600 rats were used in these experiments. A typical illustration is given in each group, but it is not clear how many rats showed the gastric changes described. Roffo found the most common lesion to be ulceration in various forms and degrees in the glandular stomach and forestomach. The lesions in the glandular stomach are well illustrated. Some were regarded as ulcers in which carcinomatous degeneration had taken place.

In a more recent publication Roffo<sup>42b,c</sup> reported the results of feeding rats various animal fats (beef, pork or lamb) or olive oil heated to 350 C for a half hour. This procedure is reported to have changed the cholesterol to oxysterol, which was found to have a fluorescence spectrum similar to some of the carcinogenic hydrocarbons. In other respects the experimental method was identical to that of the previous experiment. Malignant lesions of the stomach, liver and lung are described. Lesions of the glandular stomach far outnumbered all others, and the discussion will be limited to these. The gross findings in 200 rats are tabulated and show that over one third had some gastric lesion, most commonly multiple ulcers of the glandular stomach. In some rats gastric tumors (or polyposis) were present. Histologically, in addition to the ulcers, hyperplasia of the gastric mucosa, epithelial cysts (cystadenomas) and atypical glandular epithelium were described, and in some instances destruction of parts of the muscularis mucosae and of the muscularis was present. These changes are well shown in numerous photographs of gross specimens and in photomicrographs. Some of the more advanced lesions were regarded as adenocarcinomas.

41 Fieser, L. F. *Am J Cancer* **34** 37, 1938.

42 Roffo, A. H. (a) *Ztschr f Krebsforsch* **47** 473, 1938, (b) *Bull Assoc franç p l'étude du cancer* **28** 556, 1939, (c) *Bol Inst de med exper para el estud y trat d cancer* **15** 407, 1938.

Roffo used a total of 1,600 rats in cholesterol feeding experiments of one type or another. The number of lesions regarded as carcinomatous degeneration of ulcers or adenocarcinomas is not entirely clear, but certainly there were quite a few, and control experiments eliminated the possibility that these were coincidental. On the basis of the changes shown in the photomicrographs, the question may be seriously raised as to whether these lesions were really malignant. Aside from the profound changes in the glandular epithelium, some of the strongest evidence in favor of malignancy is found in the fact that in some cases the muscularis was destroyed in places by a downgrowth of atypical glandular tissue, cystadenomatous in form. The question arises whether a process limited in all cases by the gastric serosa can be considered malignant. In the absence of any mention of a metastasis from a gastric lesion or of an instance of invasion of a neighboring structure or organ, the evidence for malignancy is not entirely convincing. In this connection it is interesting to compare the photomicrographs of lesions regarded as adenocarcinomas by Roffo with the less severe but similar changes observed by Bullock and Rohdenburg<sup>16</sup> in rats after introducing into the stomachs cork balls with protruding pinpoints.

Waterman<sup>43</sup> fed tarréed mice cholesterol oleate twice a week. The mortality was high. Forty mice survived sixty days or more. Twelve of these had benign forestomach papillomas, and 3 had what was interpreted as squamous cell carcinoma of the forestomach. The latter were found in mice that succumbed ninety-three, two hundred and forty-eight and two hundred and forty-eight days, respectively, after the feeding of cholesterol oleate. No further description is given. Metastases are not mentioned, presumably there were none. A low power photomicrograph illustrating a carcinoma shows a papilloma near the limiting ridge. No invasion of the muscularis can be seen. On the basis of this illustration it is difficult to differentiate the lesion from previously described benign papillomas. Waterman in another experiment fed 6 untarred mice cholesterol oleate three times a week. After three hundred and eighteen days he found a malignant papilloma in the forestomach and a fibrosarcoma in the neck of the stomach in 1 mouse. Another had a squamous cell carcinoma of the forestomach after four hundred and forty-one days. A third mouse had polypoid growths in the glandular stomach showing many mitoses after three hundred and sixty days. No metastases are mentioned, and no description of the lesions are given, although photographs of the gross and microscopic appearance accompany the report. These photographs are in no way convincing. A fourth mouse after four hundred and forty-one days was found to have

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43 Waterman, N. *Acta cancerol* 2 375, 1936



a lesion regarded by Waterman as an early adenocarcinoma of the stomach without a metastasis. This is a most interesting interpretation. A description and illustrations of the lesion are given. It appears to be along the limiting ridge, depressed in relation to the surrounding mucosa, and might easily be overlooked on gross examination. The muscularis mucosae is not distinct. There is no invasion of the muscularis. A higher power photomicrograph taken from an unspecified part of the mucosa shows an acinar arrangement of apparently single layers of cells. The description and illustrations indicate there was some alteration in a small part of the glandular mucosa. That this represented a malignant change is, however, a matter of opinion not well supported by the evidence.

(d) 3,4-Benzpyrene. Oberling, Sannie, Guérin and Guérin<sup>44</sup> fed 20 mice 3,4-benzpyrene in lard once a week. Sixteen mice succumbed at the end of six months. One of these showed benign hyperplasia of the forestomach mucosa. None of the others had any appreciable gastric changes. Of 20 rats fed benzpyrene in lard, 10 died within seven months. None of these had any significant gastric lesion. The other 10 rats appeared in good health at the time of the report.

Ilfield<sup>37</sup> obtained negative results (at four months) by implanting 5 per cent benzpyrene in cholesterol pellets under the gastric serosa of ferrets.

Waterman<sup>43</sup> fed 6 mice 0.4 per cent 3,4-benzpyrene in lard daily for periods ranging from one hundred and twelve to three hundred and thirty-six days. When benzpyrene was given in watery colloidal solution, no gastric lesions were observed. With lard as the solvent, however, 5 mice had forestomach tumors that were regarded as squamous cell carcinoma. Metastases to the portal glands, peritoneum, liver, spleen or lungs were reported in 4 of these animals, but the several photographs of the lesions, including one of a reported metastatic nodule in the liver, are not convincing, and no microscopic description of the primary or metastatic lesions is given.

Rusch, Baumann and Maison<sup>45</sup> reported a low grade adenocarcinoma of the stomach in 1 of 5 rats fifteen months after injection of 3,4-benzpyrene into the submucosa of the pyloric area. A tumor, 1 by 1 cm, which had invaded the muscularis was found. There were no metastases. No further description or illustration is given. A myoma and a spindle cell sarcoma were found in 2 of the other rats.

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44 Oberling, C., Sannie, C., Guérin, M., and Guérin, P. *Bull. Assoc. franç. p. l'étude du cancer* **25**: 156, 1936.

45 Rusch, H. P., Baumann, C. A., and Maison, G. L. *Arch. Path.* **29**: 8, 1940.

(e) Methylcholanthrene Van Plohaska, Brunschwig and Wilson<sup>40</sup> gave 15 mice 2 minims (0.12 cc) of a 1 per cent solution of methylcholanthrene in olive oil orally three times a week. Eight mice survived for six months and were killed when they appeared weak and emaciated. Two of these had benign squamous cell papilloma of the forestomach at the end of one hundred and sixty-four and one hundred and seventy-five days, respectively. Four of the others had squamous cell carcinoma of the buccal mucosa or of the skin about the mouth. When a 1 per cent solution of methylcholanthrene in lard was injected into the mouths of 33 mice every other day for four months, no benign or malignant gastric lesion was found. One mouse had a squamous cell carcinoma of the angle of the mouth after sixty-seven days and another a hypopharyngeal squamous cell carcinoma after one hundred and forty days. These authors pointed out the close relationship of methylcholanthrene to the bile acids which normally bathe a large portion of the gastrointestinal tract. They conclude, however, that methylcholanthrene administered orally does not have a marked carcinogenic effect on the alimentary canal even in the upper portion lined by squamous epithelium. They suggest that certain mechanical factors may explain this in part, in addition to an apparent high degree of resistance of the epithelium of the stomach and intestine. On cutaneous application and subcutaneous injection of carcinogens, prolonged contact with the cells is possible, whereas in the alimentary canal the mucosa is constantly washed by various fluids and is, moreover, covered and perhaps protected by a layer of mucus.

Necheles<sup>46</sup> injected 250 mg of methylcholanthrene into the submucosa of the anterior wall of the stomach near the proximal border of the antrum in a dog. Three gastric biopsies taken in the region of the injection over a period of nine months have shown an inflammatory reaction but no evidence of malignant change. The experiment is still in progress.

Stewart<sup>5b</sup> reported squamous papilloma of the stomach in 4 and squamous cell carcinoma of the stomach in 4 of 30 strain A male mice which when 3 months old received injections of a solution of methylcholanthrene in mineral oil into the anterior wall of either the glandular stomach or the forestomach. All the tumors were forestomach lesions and are excellently described and illustrated. The four carcinomas were visible, one was adherent to the liver. They were located in the anterior wall except one, which was some little distance from the point of injection, at the posterior inferior margin of the forestomach. Microscopically, the infiltrating epithelium invaded all layers of the stomach and was composed of basal cells, prickly cells and flat squamous cells,

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46 Necheles, H. Personal communication to the authors.

atypical in size, shape and staining. There were numerous mitotic figures. In one tumor there were nests of epithelium within thin-walled vessels, either lymph vessels or blood vessels, between the two muscle layers of the stomach. In the case in which the abscessed liver was adherent to the wall of the stomach opposite the tumor, masses of keratin were observed within the abscess. In 2 cases small nodules composed of tumor cells were adherent to the external surface of the peritoneum. One tumor was successfully transplanted to 4 of 6 strain A mice, which had large tumors at the sites of inoculation after three weeks. Transplants of these in turn were successful in 2 of 6 mice, which had large tumors after one month. The tumors developing from the transplants were identical in morphologic characteristics to the original tumor of the stomach. In the literature on successfully induced squamous cell carcinoma of the stomach, this is one of the few reports accompanied by clear pathologic descriptions, sufficient relevant details, and photomicrographs to show the significant changes.

Stewart has indirectly raised several points of interest. Invasion of the muscularis was taken as the criterion to differentiate carcinoma from papilloma. The absence of such invasion has usually been regarded as one of the chief objections to classifying certain experimental gastric lesions as malignant. Stewart has illustrated such invasion of the muscularis and the presence of nests of epithelium within thin-walled vessels, either lymph vessels or blood vessels. The latter is usually considered a reliable indication of malignancy in spontaneous tumors. Successful transplantation of gastric tumor tissue has rarely been demonstrated before. These points will be discussed in more detail later. The possibility of coincidence is entirely eliminated by the use of pure strain mice, for no similar tumor has been observed to occur spontaneously in several thousand strain A mice.

3 Miscellaneous Factors. Twort and Bottomley<sup>32b</sup> reported a squamous carcinoma of the forestomach in a mouse painted with a watery solution of a mixture of chrysene ammonium and sodium sulfonate for twenty weeks. The tumor invaded the liver and adjacent organs. Since this was the only mucous membrane tumor in a large number of mice (12,000) subjected to similar procedures, it was considered to be spontaneous.

Hormonal substances have been of considerable importance in some fields of experimental induction of tumors, however, gastric lesions apparently have been rarely found. Pierson<sup>48</sup> reported a growth resembling mammary gland in the wall of the stomach of a castrated rabbit which had received 0.1 mg. of an estrogen twice a week for three years. In the thickened gastric wall were numerous infiltrating tubules

47 Footnote deleted

48 Pierson, H. *Ztschr. f. Krebsforsch.* 48: 177, 1938

and ducts filled with a material staining pink with eosin. This glandular tissue was nowhere seen to be related to the gastric epithelium in serial sections. There was extensive squamous cell metaplasia. This was the only such tumor found in a large group of rabbits receiving the estrogen.

Domagk<sup>49</sup> reported a gastric adenocarcinoma in 1 of 20 mice fed a diet of rice and 20 per cent olive oil, alternating each week with the usual diet, for one year. The tumor was visible grossly. Microscopically, it consisted of atypical glandular epithelium with numerous mitotic figures and extended through the muscularis mucosae and the muscularis but was limited by the serosa. There were associated inflammatory changes in the gastric wall. No metastasis is mentioned. Photomicrographs of this tumor show the changes described. Gastric polyposis, regarded as precancerous, was found in some of the other mice. A pulmonary nodule in 1 mouse was interpreted to be a metastasis from the stomach, although the gastric lesion was apparently considered precancerous. There is no photograph or description of the pulmonary nodule. No similar gastric lesion was found in 20 mice that were not fed olive oil.

#### COMMENT

*Nomenclature*—The term "experimental gastric carcinoma" almost always refers to squamous cell carcinoma of the forestomach. In a sense this is confusing, since carcinoma of the stomach in man implies adenocarcinoma. There is no experimental evidence to suggest that the two types are related except in the anatomic location of both in the stomach. Occasionally the term "experimental gastric carcinoma" has been applied to malignant tumors of other organs transplanted to the gastric wall. The use of the phrase in this sense is misleading.<sup>50</sup>

*Sarcoma*—Reports of experimental sarcoma of the stomach are uncommon and have not been included in this review. Brunschwig<sup>51</sup> found large fibrosarcomas in 2 of 3 male rats exposed for an indeterminate length of time to various carcinogenic agents. Each of these animals also had a benign fibroadenoma of the breast.

*Adenocarcinoma*—There are in the literature relatively few claims<sup>52</sup> of the production of experimental adenocarcinoma of the stomach. On close scrutiny these reports either are not convincing or do not permit objective evaluation of the malignant nature of the changes. There is no well established case of adenocarcinoma of the stomach resulting from an experimental procedure.

49 Domagk, G. *Ztschr. f. Krebsforsch.* **48** 283, 1938.

50 Besredka, A., and Gross, L. *Ann. Inst. Pasteur* **62** 253, 1939, abstracted, *Am. J. Cancer* **37** 124, 1939.

51 Brunschwig, A. Personal communication to the authors.

52 Voronoff and Alexandrescu<sup>30</sup> Roffo<sup>42</sup> Waterman<sup>43</sup> Rusch and others<sup>45</sup> Domagk<sup>49</sup>

The fact that attempts to induce adenocarcinoma of the stomach with known carcinogenic agents have been unsuccessful raises several considerations

1 Time Factor The induction period may be considerably longer than for other experimental cancers

2 Mechanical Factors Van Prohaska, Brunschwig and Wilson <sup>40</sup> called attention to the problem of keeping the carcinogenic agent in the stomach and, even when this is accomplished, of bringing the agent into intimate contact with the secreting gastric mucosa covered with mucus. The implantation or injection of carcinogenic compounds into the wall of the glandular stomach eliminates some of the mechanical difficulties, but these methods have not yet yielded the positive results that might be expected. Although these procedures may be of considerable value, particularly in the selection of effective carcinogens feeding experiments perhaps have the advantage of being more physiologic

3 Chemical Factors Perhaps there is a carcinogenic agent, specific for the glandular tissue of the stomach. Although this is purely speculative, there is some basis for such a possibility. Shear's <sup>53</sup> studies in pure strain mice indicate that 2-amino-5-azotoluene is a specific carcinogenic compound for liver tissue. Methylcholanthrene, which is closely related to bile acids, appeared on theoretic grounds to be possibly carcinogenic for gastric glandular tissue. Reports, however, do not yet indicate that it has such an action when given by mouth (Van Prohaska, Brunschwig and Wilson <sup>40</sup>) or when injected into the gastric wall (Stewart <sup>5b</sup>)

4 Species or Strain Susceptibility Experimental cancer may at times be induced in an organ or tissue of an animal while the same procedure in other animals of a closely related species or strain has no carcinogenic effect. The well known difference in the response of rats and rabbits to tarring is a classic example. Relatively few species have been used in attempts to induce gastric adenocarcinoma. The possibility that a species or strain susceptible to known carcinogenic agents may exist is not entirely excluded

5 Tissue Resistance A more basic factor may be the apparent resistance of normal gastric glandular tissue to a carcinomatous change. There is considerable pathologic evidence that carcinoma does not develop in an unaltered gastric mucosa (Konjetzny <sup>7</sup>). The question arises as to whether the known carcinogenic agents can be effective in the normal glandular stomach of an animal. Possibly preliminary benign changes are prerequisite. The more important question would then still remain as to what brings about the earlier benign changes

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<sup>53</sup> Shear, M. J. *Am J Cancer* **29** 269, 1937

*Squamous Cell Carcinoma*—It is generally accepted that squamous cell carcinoma of the forestomach has been experimentally produced in rats and mice. However the impression given at times that it has been produced rather commonly is not supported by a review of the literature. The actual number of instances on record is difficult to estimate in view of the doubtful malignancy of some induced forestomach tumors regarded as carcinoma and the questionable relation of some of these to the experimental procedure. In this regard Stewart's<sup>5b</sup> work gives promise of more successful and consistent results in the near future. One of his observations is particularly noteworthy. With methylcholanthrene the estimated average induction period of both benign and malignant squamous cell tumors of the stomach was about fourteen months. This is a considerably longer period than is needed to induce tumors of other tissues with methylcholanthrene in the same strain of mice. Although this apparently greater resistance of gastric squamous epithelium to carcinogenic action is not definitely known to exist in all animals and for all carcinogenic compounds, the numerous negative results obtained in experiments of shorter duration strongly suggest this possibility.

In weighing the evidence of malignancy, the presence of a mass of atypical or keratinized squamous cell tissue or of a large cavity filled with caseous material in the lung of rats usually does not indicate a metastasis. It is so frequently found that not much significance can be attached to it. Bullock and Rohdenburg<sup>16</sup> called attention to this fact in 1918 and in an accompanying photomicrograph showed squamous cell epithelium with mitotic figures in the lung of a rat with bronchopneumonia. Bonne<sup>9b</sup> later found a large keratinized squamous mass in the periphery of the lung of a rat dying of bronchopneumonia. No connection with a bronchus could be found. There was no squamous cell tumor elsewhere, and it was interpreted to be a metaplastic mass primary in the lung. Wolbach and Howe<sup>21</sup> and others found widespread squamous cell metaplasia in vitamin A-deficient rats. None, however, have shown the presence of squamous cell metaplasia and bronchiectasis in rats as convincingly as Passey, Leese and Knox<sup>13c</sup>. These workers found that 51 per cent of a large group of laboratory rats had bronchiectasis in some degree or other, which occurred independent of the diet. Some bronchiectatic cavities filled with purulent material replaced several lobes of the lung. Squamous cell metaplasia of the bronchial tree was also common and independent of the diet, although it was more marked in vitamin A-deficient rats. Keratinization of these metaplastic masses was almost exclusively limited to the vitamin A-deficient animals. In 1 rat and 2 mice squamous cell metaplasia of the alveoli was found. These lesions are excellently illustrated. There

is no photomicrograph in the literature of a so-called metastasis to the lung from a squamous cell tumor of the forestomach that cannot be matched among those accompanying the paper of Passey, Leese and Knox. These authors considered the possibility of several etiologic factors without reaching a definite conclusion. It is certain, however, the lesions are not metastases. This is confirmed by many unpublished observations (Steiner<sup>54</sup>).

Abdominal metastases have apparently not been so confusing. Enlarged abdominal lymph nodes obviously do not indicate metastasis in all cases. In the presence of severely infected benign forestomach changes, it might be surprising if the regional lymph glands were not enlarged. For this reason illustrations of such enlargements are not convincing unless accompanied by photomicrographs showing the metastatic tissue. Likewise there are causes other than carcinomatous invasion of neighboring structures to account for adhesions between the stomach and these organs.

Evidence in favor of malignancy could be shown by irreversibility of experimental gastric lesions simulating carcinoma. Obviously it is not always possible to demonstrate this, especially when the carcinogenic agents are injected into the gastric wall. In the case of dietary deficiencies or the feeding of carcinogenic agents this method seems applicable. Bonne's<sup>9b</sup> report of a squamous cell carcinoma in a mouse which had received tar by mouth for thirty-three weeks and which died almost five months later shows that the method is feasible. Another example is the experiment of Hoelzel and DaCosta<sup>23b</sup> in which they used dietary means to induce forestomach lesions in rats, which almost disappeared when a complete diet was resumed.

Transplantation of squamous cell tumors of the forestomach has not been attended by much success. This may have been due to (1) infection of the implants, as Fibiger and others have emphasized, (2) the nonmalignant nature of some of the tumors, (3) the large amount of acellular and keratinized material present or (4) the difficulties in transplanting tissue from one strain of animals to another. Fibiger<sup>11c</sup> apparently succeeded in the case of a mouse. Metastases to lymph nodes were transplanted into 28 of 55 mice that survived inoculation, representing four generations of "takes" covering a period of one year. The transplanted tissue maintained the histologic appearance of the original tumor of the stomach. Slye, Holmes and Wells<sup>4</sup> in the case of two spontaneous gastric carcinomas in mice obtained only a few takes with one of the tumors, which could not be carried to a second generation. Stewart<sup>5b</sup> transplanted an induced squamous cell tumor of the stomach of a mouse through two generations. Microscopically, the transplants were identical to the original tumor.

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<sup>54</sup> Steiner, P. E. Personal communication to the authors.

In this connection the experience with transplantation of induced squamous cell tumors of the skin deserves consideration. Woglom<sup>55</sup> in a comprehensive review of experimental tar cancer stated that spontaneous keratinizing neoplasms had long been known to be refractory to propagation and that similar difficulties were encountered with induced squamous cell tumors even when they gave definite evidence of being malignant in other ways. The failure to obtain "takes" with such tumors cannot, therefore, be regarded as eliminating the possibility of malignancy. On the other hand, a positive result might be due merely to temporary proliferation of the grafts. In either case the result might be equivocal. Murray and Woglom (cited by Woglom<sup>55</sup>) found autotransplants in mice more satisfactory than homotransplants. Normal tissues or benign growths proliferated only temporarily or not at all, whereas malignant growths gave a high percentage of positive results. In the hands of these observers autotransplantation was sometimes successful with tar tumors that appeared histologically benign at the time of implantation. This was regarded as evidence that autotransplantation could at times facilitate the recognition of a malignant tumor before histologic examination. Kreyberg<sup>56</sup> was unable to confirm the observation that tumors appearing histologically benign in mice were autotransplantable.

Rous and Kidd<sup>57</sup> noted that autotransplants of a virus papilloma in rabbits in some instances grew while the original papilloma retrogressed. This was found particularly when inflammation of bacterial origin ensued at the site of inoculation.

These examples illustrate the difficulties encountered in interpreting the significance of both successful and unsuccessful transplants of induced squamous cell tumors of the skin. Since these experimental tumors are the ones most analogous to those of the forestomach, the same uncertain interpretation may apply to the results of transplantation of the latter as well.

*Criteria of Malignancy*—The question of what characterizes an experimental carcinoma has been a source of controversy. Fibiger<sup>14c</sup> set down clearly his criteria of malignancy with respect to squamous cell tumors of the forestomach and supported his opinion by pointing out the similarity of such changes to those occurring in spontaneous squamous cell carcinoma in man and animals. These criteria were

1 Heterotopic downgrowth of epithelial cells, not only the normal type of the basal epithelial layers, but, mixed with these, atypical and keratinized cells in abundance, partly arranged as spherical masses and horny globes

55 Woglom, W. H. Arch Path **2** 533 and 709, 1926

56 Kreyberg, L. J. Cancer Research **9** 381, 1925

57 Rous, P., and Kidd, J. G. J. Exper. Med. **69** 399, 1939



2 Infiltrative growth of these heterotopic and atypical epithelial cells into the deeper layers, splitting up invasively the elements of the connective tissue of the mucosa and the muscle cells of the muscularis mucosae, forming isles and spurs in the latter—as most frequently seen—also penetrating through this membrane into the superficial or deeper layers of the submucosa

Bullock and Rohdenburg<sup>16</sup> questioned the malignant nature of Spiroptera tumors such as Fibiger's in the absence of active invasion of the muscularis and definite metastases. Bonne placed considerable reliance on invasion of blood vessels as a sign of malignancy in 1 case<sup>9a</sup> and infiltration of the muscularis in another<sup>9b</sup>. Stewart<sup>6b</sup> differentiated papilloma from carcinoma on the basis of invasion of the outer muscular layers of the stomach. Additional evidence of malignancy was also present in one case in which nests of epithelium were found in thin-walled vessels between the muscle layers of the stomach, either lymph vessels or blood vessels, and in another the tumor was successfully transplanted to other mice through two generations.

A fundamental question is whether the microscopic criteria of spontaneous malignancy can be applied to experimental tumors in differentiating the malignant from the benign. In the final analysis, malignancy may be judged only by the course of events. Certain histologic characteristics of spontaneous lesions have become synonymous with malignancy, however, this relationship was at first empirically established. Tumors following a malignant course were found to have some differentiating morphologic characteristics. Earlier and earlier stages of these characteristics were recognized, so that now it is often possible to predict that a spontaneous tumor will follow a malignant course on the basis of its structure only and without any definite proof of malignant activity beyond a few microscopic changes which, if they did not progress, could not be classified as malignant and would not interfere with the well-being of the organism. If early morphologic changes from the accepted normal are used to predict a malignant sequence, they are reliable guides only so far as this sequence has been shown always to follow. It cannot be inferred with certainty that the histologic criteria established empirically as pointing to an irreversible and progressive process in a spontaneous tumor necessarily apply to induced tumors or other forms of spontaneous tumors. If a malignant alteration represents an intrinsic irreversible cellular change, manifested by aggressive and destructive invasion of other tissues and organs and continuing until the death of the organism, some of the criteria of malignancy applied to induced tumors may at times be inadequate.

These remarks in relation to experimental squamous cell carcinoma of the forestomach can become the subject of futile controversy. However, if detailed and careful studies of induced squamous cell tumors

of the skin, such as those of Yamagiwa and Ichikawa<sup>58</sup> and Ichikawa and Baum<sup>59</sup> and the comprehensive reviews of Woglom<sup>55</sup> and Seelig and Cooper,<sup>60</sup> are again consulted, some light may be thrown on the problem

Murray and Woglom (cited by Woglom<sup>55</sup>) made use of four criteria of malignancy "(1) the progressive growth of a tumor after painting has been discontinued, and the growth of its autotransplants, (2) the recurrence after wide excision, (3) histologic evidence of local infiltrative growth, and (4) metastases. They regarded the loss of differentiation and the presence of atypical cellular characteristics as wholly inadequate." These investigators apparently found that simpler criteria of induced malignancy did not distinguish benign from malignant tumors.

Rous and Kidd<sup>57</sup> described squamous cell tumors in rabbits following repeated tarring of the ear which have all the morphologic appearances of carcinoma, including extension through lacunae in the cartilage and presence of proliferating epithelium in the lymphatics. These tumors were at times morphologically indistinguishable from carcinoma and were called carcinoids. When the tarring was discontinued, the carcinoids retrogressed, whereas the carcinomas continued to show active invasive properties. If the tarring was continued without interruption, the carcinoids eventually ceased to grow destructively and took on the gross forms of old papillomas. These investigators also mentioned a group of tumors which had active malignant properties only as long as tarring was continued.

On the basis of these and numerous similar observations in regard to experimental squamous cell tumors of the skin, it is questionable whether the morphologic characteristics of spontaneous squamous cell carcinoma in man or animals can be applied to induced gastric tumors unless *the latter continue to show malignant activities in the absence of the agent used to bring them about*. Fibiger believed he demonstrated such an irreversible change, but the factor of a dietary deficiency was not eliminated in his experiments. Since some dietary deficiencies in themselves apparently lead to forestomach changes meeting Fibiger's criteria of malignancy, the validity of his interpretation is open to question. In view of this there is obviously some hesitancy in agreeing that all experimental squamous cell tumors of the forestomach regarded in the literature as carcinoma do actually represent malignant alteration of tissues.

What criteria would serve to differentiate induced benign and malignant gastric tumors cannot be settled at present. More of these tumors

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58 Yamagiwa, K., and Ichikawa, K. J. Cancer Research **3** 1, 1918

59 Ichikawa, K., and Baum, S. M. J. Cancer Research **9** 85, 1925

60 Seelig, M. G., and Cooper, Z. K. Am. J. Cancer **17** 589, 1933

will have to be observed carefully in order to determine their own peculiarities. It is not known, for example, whether such carcinomas are more apt to metastasize than they are to invade neighboring organs, or whether invasion of the musculature indicates a definitely irreversible process. Induced cancers should, however, have those characteristics generally considered inherent in a malignant growth: (1) the ability to proliferate independently as metastases, (2) the ability to invade progressively and destructively neighboring tissues and organs, (3) irreversibility of these properties in the absence of the extrinsic factor initially held responsible for the cellular change. Since such changes have been observed to occur in spontaneous squamous cell carcinoma of the forestomach in mice (Slye, Holmes and Wells,<sup>4</sup> Wells, Slye and Holmes<sup>3</sup>) there should be, in addition, (4) reasonable evidence to indicate a causal relation of the experimental procedure to the tumor.

Application of these criteria to induced gastric tumors is not always easy, but unless they can be demonstrated, the malignant nature of the tumor cannot be considered as proved. The histologic appearance of the tumor alone is not adequate.

#### SUMMARY

Spontaneous gastric adenocarcinoma is found rarely in animals and for all practical purposes can be said to occur exclusively in man. Consequently studies of this most important human malignant lesion are limited for the present to clinical material. A review of the literature discloses no reliable method of inducing adenocarcinoma of the stomach in animals; in fact, there is no well established case of an adenocarcinoma of the stomach produced experimentally.

Some success has attended efforts to induce squamous cell carcinoma of the forestomach in mice and rats, although considerably less than the claims in the literature would indicate. There is no experimental evidence to suggest that the two types of gastric carcinoma are related except in the anatomic location of both in the stomach.

The criteria of induced malignancy are considered. Proof of malignancy is not given by the histologic appearance of a tumor, whether spontaneous or induced, but by the demonstration of malignant activity as evidenced by (1) the ability to proliferate independently as metastases, (2) the ability to invade progressively and destructively neighboring tissues and organs, (3) irreversibility of these activities, which must be shown to continue in the absence of the extrinsic factor initially held responsible for the cellular changes. It must, furthermore, be shown that (4) a given malignant change occurs with sufficient regularity to establish a causal relationship of the experimental procedure to the cancer.

## Notes and News

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**University News, Promotions, Resignations, Appointments, Deaths, Etc**—Alfred H W Caulfeild, research member of the Connaught Laboratories, Toronto, Canada, died on May 2, at the age of 59

Esmond R Long, Philadelphia, has been elected a member of the American Philosophical Society

Valy Menkin has been advanced to the rank of assistant professor of pathology at the Harvard Medical School

The Rockefeller Foundation has made a grant of \$15,000 toward the development over a three year period of legal medicine at Harvard Medical School

The George M Kober Medal of the Association of American Physicians for 1940 has been presented to F F Russell for his introduction of double sugar medium for the cultivation of typhoid bacilli, and the same medal for 1941 has been awarded to William deB MacNider for his study of acquired resistance of tissue cells after injury to the liver and kidney

The old autopsy house of the Philadelphia General Hospital ("Old Blockley"), which has been restored and named the Osler Memorial Building, was dedicated on June 8. At the same time the painting "Osler at Old Blockley" by Dean Cornwell was shown

Louis Hamman, Johns Hopkins University, has been elected president of the Association of American Physicians

The Ettore Marchiafava Prize, founded by the University of Rome in 1938 for the best work on morbid anatomy or general pathology, has been awarded to Mario Monacelli, director of the dermatologic clinic of the University of Messina, and Giulio Raffaele, of the University of Rome

**Society News**—The Biologic Photographic Association will meet at the Hotel Schroeder, Milwaukee, Sept 12 to 14, 1940. The address of the secretary is Magee Hospital, Pittsburgh

# Abstracts from Current Literature

TO SAVE SPACE THE ORIGINAL TITLES OF ABSTRACTED ARTICLES SOMETIMES  
ARE SHORTENED

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## Pathologic Chemistry and Physics

INCREASING PLASMA PROTHROMBIN IN THE NEWBORN INFANT L B SHETTLER,  
E DELFS and L M HELLMAN, Bull Johns Hopkins Hosp 65 419, 1939

The plasma prothrombin level of the newborn infant can be raised not only by feeding vitamin K concentrate directly to the infant after birth but also by administering it to the mother prior to delivery. The values obtained by administration of the concentrate to the mother are often three times those normally seen and in general are higher than those which can be attained by administering vitamin K concentrate to the baby after birth.

FROM AUTHORS' SUMMARY

AN ELECTROPHORETIC STUDY OF NEPHROTIC SERUM AND URINE L G LONGSWORTH and D A MACINNES, J Exper Med 71 77, 1940

The electrophoretic patterns of the serum and urine of 2 patients with lipid nephrosis have been obtained and have been compared with a typical pattern of normal serum. The patterns of the pathologic serums deviated widely from the normal, albumin was relatively low and globulin high. The comparison of the patterns of nephrotic serums cleared by centrifugation and by ether extraction shows that a large portion of the beta globulin consisted of a labile lipoprotein. The pattern of the proteins of the nephrotic urine resembled that of the proteins of normal serum, with, however, significant differences.

FROM AUTHORS' SUMMARY

VITAMIN K E A DOISY and others, Science 91 58, 1940

During the decade following Dam's first observations on the hemorrhagic syndrome the combined efforts of several groups of investigators have solved many of the important problems connected with the new vitamin. Sources of vitamin K were discovered, methods of extraction and purification devised, the isolation effected, the structure of  $K_1$  worked out and then verified by synthesis, and a promising start made on the therapeutic applications. In addition, simple water-soluble compounds with antihemorrhagic properties have been supplied for clinical work. Preliminary results with these compounds are encouraging.

FROM AUTHORS' SUMMARY

ADRENALIN CONTENT OF THE ADRENAL GLANDS IN DIPHTHERIA INTOXICATION C A ASHFORD, Brit J Exper Path 20 385, 1939

Ashford has estimated the adrenalin content of the adrenal glands in diphtheria intoxication to determine whether the content is reduced during the acute stages of diphtheritic toxemia. Reports on this point, based mainly on histologic methods applied to experimental animals and human postmortem material, have been conflicting and a number of theories of the circulatory collapse have been built up on the assumption of a depletion or an exhaustion of the medullary adrenalin. Guinea pigs have been used, and the results show that reduction of adrenalin content to any marked extent is not found. Histologic observations are reported briefly, and the possible significance of cortical damage discussed.

### Microbiology and Parasitology

TOXOPLASMIC ENCEPHALOMYELITIS A WOLF, D COWAN and B H PAIGE,  
Am J Path **15** 657, 1939

A fifth case of a new disease, granulomatous encephalomyelitis due to a protozoon, occurring in an infant is described. The clinical and pathologic observations in this case are shown to be similar to those in the first 4 cases. This group represents a distinct disease entity. The disease affects young infants, produces manifestations of general involvement of the nervous system, may give rise to ophthalmoscopically identifiable focal lesions in the euegrounds and terminates fatally after an acute or subacute course. The spinal fluid shows xanthochromia, a high protein content and pleocytosis. The central nervous system is the site of focal inflammatory and degenerative lesions, which are widely disseminated. Similar changes are found in the retina and choroid. Miliary granulomas are a characteristic feature of the process in the nervous system. Focal inflammatory lesions were present in the heart and striated muscle in 1 case. A protozoan parasite is present in all the lesions. The results of transmission of the infection to animals from the case reported here indicate that the causative protozoon is a *Toxoplasma*. The designation *Toxoplasma hominis* is suggested for the microorganism and the term "toxoplasmic encephalomyelitis" for the disease.

FROM AUTHORS' SUMMARY

BACTERIOSTATIC AND ANTITOXIC ACTIONS OF SULFANILAMIDE JOSEPH T KING,  
AUSTIN F HENSCHEL and BERYL S GREEN, J A M A **113** 1704, 1939

A tissue culture study has been made of the bacteriostatic and antihemolytic properties of sulfanilamide. All strains of beta streptococci studied were inhibited. The bacteriostatic effect varied directly with the concentration of the drug and inversely with the number of bacterial colonies. Reduction in hemolysis was observed to accompany bacteriostasis. Correlation of the reduction in hemolysis with the observed bacteriostasis leads to the conclusion that the antihemolytic effect is secondary to bacteriostasis. The drug regularly inhibits the wide, diffuse peripheries usually seen around colonies of beta streptococci growing in clots of tissue culture. The drug causes the development of abnormal, long chains of streptococci.

FROM AUTHORS' SUMMARY

HEMOLYTIC STREPTOCOCCUS LYMPHADENITIS IN GUINEA PIGS C V SEASTONE,  
J Exper Med **70** 347, 1939

A group of guinea pigs carrying a chronic streptococcic cervical lymphadenitis has been studied. The chronic disease may be transmitted with pure cultures of streptococci isolated from the naturally occurring abscesses. Its probable mode of transmission under natural conditions was shown to be by ingestion of the infective agent. The spontaneous appearance of an acutely fatal variant was observed. Infection with the chronic strains protected animals against the highly virulent strain. Such immunity could not be passively transferred to either mice or guinea pigs, nor could any opsonizing, precipitating or bactericidal antibody be associated with it. Allergy could not be correlated with this immunity. The dissociation of the chronic and acute strains was investigated, and organisms in noninvasive phases were isolated. No precipitin reaction attributable to an antigenic virulence factor could be demonstrated. No protection was obtained with vaccines or aggressins.

FROM AUTHOR'S SUMMARY

VIRULENCE OF GROUP C HEMOLYTIC STREPTOCOCCI C V SEASTONE, J Exper  
Med **70** 361, 1939

A nonantigenic mucoid polysaccharide similar to that described by Kendall, Heidelberg and Dawson was isolated from group C hemolytic streptococci.

A simple method for its quantitative estimation is described. By means of this method, as well as by the direct isolation of the carbohydrates, the size and persistence of capsules in young cultures of various strains have been related to the nonantigenic mucoid polysaccharide.

PURITY OF PREPARATIONS OF ELEMENTARY BODIES OF VACCINIA J. E. SMADGL, T. M. RIVERS and E. G. PICKELS, *J. Exper. Med.* **70** 379, 1939

A method of estimating the purity of preparations of elementary bodies of vaccinia is described. It depends on the comparison of the number of infective units of virus in a given material with the number of elementary bodies. The latter figure is estimated from the dry weight of the preparation by means of a calculated value for the weight of a single dehydrated elementary body. The ratio of the number of infective units of vaccine virus to the number of elementary bodies varied between 1.24 and 1.92 in seven consecutive experiments, the average was 1.42. These ratios indicate a high degree of purity of the preparation. Moreover, they indicate that a relatively high percentage of the elementary bodies in the preparations was infective.

FROM AUTHORS' SUMMARY

MALIGNANT PANLEUKOPENIA OF CATS W. D. HAMMON and J. F. ENDERS, *J. Exper. Med.* **70** 557, 1939

The most conspicuous clinical finding in the course of this virus disease is fulminating panleukopenia. The earliest significant decrease noted is usually in the number of the lymphocytes. From the study of lymph nodes and bone marrow during the period of incubation and throughout the illness, it appears that a failure of leukopoiesis is the cause of the leukopenia. Inclusion bodies in the primitive blood cells of the marrow suggest a direct action of the virus on these cells. When recovery occurs, a marked myelogenous leukemoid response is noted. Available data indicate the presence of mild anemia due to a failure in erythropoiesis, less marked than the leukopenia, probably because of the longer life of the adult circulating erythrocytes. The erythrocytes appear to have increased fragility, and the serum has a slight increase in icterus, suggesting an increased mean erythrocyte age. During recovery erythropoiesis does not begin until after the myeloid marrow response has begun to subside, possibly because of previous mechanical crowding of the marrow by the more rapidly growing myeloblasts and myelocytes.

FROM AUTHORS' SUMMARY

INCLUSION BODIES IN SCARLET FEVER J. BROADHURST and others, *J. Infect. Dis.* **64** 193, 1939

That a virus is concerned in the production of scarlet fever is indicated by the presence of inclusion bodies in the tissues of the upper respiratory area of patients with scarlet fever, the presence of inclusion bodies in the white blood corpuscles of such patients, the presence of inclusion bodies in serial tissue cultures inoculated with bacteria-free filtrates of blood from such patients.

FROM AUTHORS' CONCLUSIONS

BILE ACTION ON PNEUMOCOCCI AND STREPTOCOCCI P. H. GREY, *J. Infect. Dis.* **64** 206, 1939

*Pneumococcus* and *Streptococcus viridans* on blood agar alter the red cells surrounding their colonies so that these cells are resistant to the hemolytic effect of bile. Dried bile placed on the blood agar colonies of *Pneumococcus* dissolves the colonies but leaves the zone of fixed cells intact. Colonies of *Str. viridans* and *Streptococcus haemolyticus* are not dissolved. This is therefore a simple means of differentiating these colonies.

FROM AUTHOR'S SUMMARY

VIABILITY OF TUBERCLE BACILLI ON RESTRICTION OF OXYGEN T S POTTER, J Infect Dis **64** 261, 1939

Human tubercle bacilli, like avian, may survive prolonged drastic deprival of oxygen at 37 C. Avian bacilli, like the human type, studied by Novy and Soule, are unable to survive a relatively mild restriction of oxygen at 37 C when such restriction is combined with an accumulation of the gaseous products of their metabolism on glycerin agar

FROM AUTHOR'S CONCLUSIONS

THE NUTRITION OF CORYNEBACTERIUM DIPHTHERIAE W C EVANS, W R C HANDLEY and F C HAPPOLD, Brit J Exper Path **20** 396, 1939

Evans, Handley and Happold, continuing their studies on the nutritional requirements of *Corynebacterium diphtheriae* types, find that the additional growth factor present in tissue extractives required by certain gravis strains is pantothenic acid. The mitis and some gravis strains which had previously been grown in chemically defined mediums synthesize this "gravis growth factor". Pantothenic acid is a compound of beta-alanine and a dihydroxyvaleric acid, according to current literature, this substance is identical with the chick antidermatitis factor and is one of the B complex vitamins. The paper also describes experiments which show that strains of *C. diphtheriae* grown in chemically defined mediums synthesize substances with physiologic activities similar to those of aneurin, riboflavin and "co-enzymes I or II".

INFLUENZAL BRONCHITIS M STRAUB, J Path & Bact **50** 31, 1940

In mice the virus of influenza affects specifically the epithelium of the respiratory tract from the bronchioles to the bifurcation of the trachea. It causes catarrhal bronchitis with collapse of lung tissue. This collapse is merely a complication of the epithelial process and is not in itself specific. It may be absent if a weak virus has been used or if the mice are adults. In such circumstances treatment with diluted diphtheria toxin prior to infection or treatment with Tyrode solution one or two days after infection is capable of aggravating the consequences of the diffuse catarrhal inflammation. In influenzal bronchitis, after a degenerative stage there follows a regenerative stage of the epithelial process. In the larger bronchi, this entails restitutio ad integrum; in the bronchioles, metaplastic epithelial changes may occur. Immunity to influenza virus in mice depends largely on the presence of such epithelialized areas. Even very slight metaplastic change seems to give complete protection.

FROM AUTHOR'S SUMMARY

HISTOPHYSIOLOGY OF THE TUBERCLE E TONUTTI and J WALLRAFF, Beitr z path Anat u z allg Path **103** 78, 1939

A series of rabbits were infected by the technic of Bieling and Schwartz. They received a preliminary sensitizing injection of killed human tubercle bacilli and three weeks later an intravenous injection of living tubercle bacilli of the bovine type. During the entire duration of the experiments the animals were maintained on a normal diet supplemented with vitamin C. The histiocytes and epithelioid cells in the tuberculous lesions situated in the lungs, adrenals and elsewhere were found to contain a striking amount of ascorbic acid by the silver nitrate technic, as well as phagocytosed tubercle bacilli. In sharp contrast, the lymphocytes contained no histochemically demonstrable vitamin C.

R J LEBOWICH

## Immunology

THE SITE OF SENSITIVITY IN THE ARTHUS PHENOMENON A R RICH and R H FOLLIS JR, Bull Johns Hopkins Hosp **66** 106, 1940

In the Arthus type of hypersensitivity there is vascular sensitivity, but the cells of the tissues at large are not sensitized. Tissue death in the Arthus reaction



results primarily from impairment of nutrition due to vascular damage and to clogging of the tissue spaces with exudate and hemorrhage. It has been shown by numerous investigators that if foreign protein is injected one or more times into the cornea of one eye a subsequent injection after some days leads to a more marked corneal reaction than occurs as a result of an injection of the protein into the opposite, untreated eye. This has been attributed to the development of local hypersensitivity in the treated eye. The present experiments indicate that the more marked reaction in the treated eye is due not to a higher degree of local sensitization of the corneal tissue but to the increased vascularity which results from the preliminary intracorneal injections, from which a greater amount of exudate can be derived more promptly.

FROM AUTHORS' SUMMARY

SEROLOGIC STUDIES ON SUGAR J M NEHI and others, J Exper Med **70** 427, 1939

Solutions of all of the chemically pure sucrose reagents of American manufacture that were tested gave reactions with type II antipneumococcus serum. The capacity of that antiserum to react with sucrose solutions was removed by absorption with the homologous pneumococci or with *Leuconostoc mesenteroides*. The serologic reactivity was due not to sucrose itself but to accompanying substances that could be removed by precipitation with a proper concentration of alcohol or by treatment with activated carbon. Although type II antipneumococcus serum was used to detect its presence, the reactive material found in the sucrose can be considered to be only related to, and not identical with, antigens of type II pneumococci.

FROM AUTHORS' SUMMARY

BACTERIAL AGGLUTINATION H E ALEXANDER and M H FIDELBERGER, J Exper Med **71** 1, 1940

The quantitative, absolute methods of agglutinin and precipitin analysis previously developed for antipneumococcus serum have been shown to be applicable to horse and rabbit serum and plasma containing antibody for influenza virus type B. With the aid of these methods and improved immunization schedules, the antibody content of the rabbit serum has been increased five to ten times. The method recommended for the purification of rabbit antipneumococcus antibody has also been found applicable to the antibody for influenza virus type B in rabbit serum.

FROM AUTHORS' SUMMARY

PERMEABILITY OF THE HUMAN PLACENTA TO ANTIBODIES A S WIENER and I J SILVERMAN, J Exper Med **71** 21, 1940

The ratio of the titer of any of the various antibodies, e. g., hemagglutinins and syphilitic reagent, in the maternal blood to that of the corresponding antibody in the umbilical cord blood was found to be relatively constant, the value falling somewhere between 8 and 16. This figure may be considered the "index of permeability" of human placenta to antibodies, or the coefficient of the distribution of antibodies between maternal and cord blood. The possible application of these findings to the study of placental permeability to sensitizing antibodies (or reagents) is discussed.

FROM AUTHORS' SUMMARY

ANTIGENIC RELATIONSHIPS OF STRAINS OF BACTERIUM NECROPHORUM P H WALKER and G M DACK, J Infect Dis **64** 285, 1939

A serologic study was made of 12 strains of *Bacterium necrophorum*—9 of human and 3 of bovine origin. Rabbits were immunized with 10 different strains of *Bact. necrophorum*. The immune serum thus produced agglutinated homologous strains in dilutions ranging from 1:160 to 1:1,280. Some heterologous strains were agglutinated to titer or nearly so by certain of these antisera.

Distinct antigenic groups were demonstrated by means of agglutination and agglutinin absorption tests. One comprised 3 strains, another 2 and a third 3. Four other strains were highly specific and probably represented other serologic groups. Virulence of these strains, as evidenced by their pathogenicity for rabbits, was no criterion for differentiating antigenic relationships, since 2 virulent strains were antigenically related to a strain of low virulence. Source of strains was not a differentiating characteristic, for of strains from chronic ulcerative colitis one was serologically related to a strain isolated from a metastatic subacromial abscess, and another, to a strain from the blood stream.

TESTS OF ANTIPNEUMOCOCCIC TREATMENTS IN RATS W. J. NUNGESTER and  
A. H. KEMPF, *J. Infect. Dis.* **64** 288, 1939

It is possible to study the effects of various therapeutic procedures for pneumonia in rats with experimentally produced pneumonic lesions. Such lesions parallel more closely the conditions encountered in man than do infections in rabbits or mice, which are primarily involvements of the blood stream with limited nonpulmonary lesions. In the rat the difficulty of favorably influencing the course of the disease was readily demonstrated. Specific immune serum decreased the incidence and mortality of pleurisy. However, a very appreciable mortality remained despite treatment.

FROM AUTHORS' SUMMARY

SEROLOGIC IDENTIFICATION OF CLOSTRIDIUM TETANI J. D. MACLENNAN, *Brit. J. Exper. Path.* **20** 371, 1939

In his study of the agglutination reactions of *Clostridium tetani* Maclellennan confirmed and extended the observations of Gunnison. He suggests the use of O anti-serum as an aid to the recognition of aberrant strains. By this means he has identified a new serologic type of *C. tetani*.

PASSIVE IMMUNIZATION TO THE VIRUS OF INFLUENZA R. HARE, *J. Path. & Bact.* **49** 411, 1939

It is possible to protect mice against intranasal infection with influenza virus by prior intraperitoneal administration of ferret immune serum. When the serum is administered after the virus, its effect depends on (a) the time after infection at which it is given and (b) the severity of the infection (number of minimal lethal doses of virus inoculated). The immune serum obtained on infection with the PR 8 strain of virus is effective against heterologous strains in passive immunity experiments. The serum of a horse immunized with influenza virus was less effective in passive immunity experiments than ferret immune serum, even in the case of concentrates which in neutralizing activity were comparable to the ferret serum.

FROM AUTHOR'S CONCLUSIONS

A STUDY OF HEMORRHAGIC PHENOMENA A. ALECHINSKY, *Ann. Inst. Pasteur* **63** 41, 1939

The Sanarelli and the Schwartzman phenomena were produced simultaneously in rabbits by a special technic and were shown to be identical. This hemorrhagic phenomenon has nothing in common with anaphylaxis. The reactions could not be elicited by horse serum, and passive transfer could not be demonstrated. The active substances which induced these reactions were found in living and dead cultures and in filtrates of various bacteria and seemed to be associated with toxins. Detailed histologic studies of the lesions produced in the Sanarelli-Schwartzman phenomena were made. The vascular changes on which the reaction depends were produced after the primary injection, but vascular rupture and thrombotic and necrotic lesions of the liver, kidneys and lungs occurred only after the second, or shocking, dose. The preparatory injection caused alterations in the hepatic

lobules and in the walls of the glomeruli, but the development of hemorrhagic and necrotic areas was uniquely connected with the shocking dose. True rupture of the endothelium of the capillaries occurred in the lung. M P LUXEN

EFFECT OF BACTERIA ON SERUMS GIVING NEGATIVE WASSERMANN REACTIONS  
O SIEVERS, *Acta path et microbiol Scandinav* **16** 365, 1939

Serums giving negative Wassermann reactions gave positive reactions from one to two days and anticomplementary reactions six days after inoculation with *Bacillus cereus*. The change became manifest earlier with cholesterolized beef heart extract than with cholesterol-free extracts. *Staphylococcus aureus*, *Escherichia coli* and *Bacillus alcaligenes faecalis* caused negatively reacting serums to become anticomplementary. *Bacillus terminosporus* had no such effect, while the growth of all the aforementioned bacteria produced anticomplementary properties in dextrose broth. The same culture medium behaved at first like a serum with a positive Wassermann reaction after inoculation with *B. cereus*, but after further incubation it acquired anticomplementary qualities. There was no noticeable relation between the serologic properties of the culture medium and the changes in the hydrogen ion concentration. I DAVIDSOHN

## Tumors

TUMOR INDUCTION AND TUMOR GROWTH IN HYPOPHYSECTOMIZED MICE. R KORTEWEG and F THOMAS, *Am J Cancer* **37** 36, 1939

In this experimental study the influence of the hypophysis on the induction of tumors and on the growth of tumors was studied in mice. When the genetic constitution of the mice was compatible, transplantation was successful in every case. The tumor grafts grew more slowly in hypophysectomized animals than in controls of the same age, but the relation of the final tumor weight to the body weight was the same in the animals operated on and controls of equal age. Papillomas and carcinomas induced by 3,4-benzpyrene appeared markedly later in the hypophysectomized mice than in the controls. With regard to the growth of tumor transplants and the response to carcinogenic agents, no qualitative difference was found between hypophysectomized mice and controls. The observed differences were entirely of a quantitative nature. FROM AUTHORS' SUMMARY

LYMPHOBLASTOMA IN MICE FOLLOWING ADMINISTRATION OF CARCINOGENIC TAR  
A M BRUES and B B MARBLE, *Am J Cancer* **37** 45, 1939

A strain of mice derived from Bagg albino stock shows occasional lymphomatosis with a subleukemic blood picture, which runs a chronic course and appears not to be transplantable into normal mice of the same or other strains. The incidence of this condition is normally 2 per cent. Following cutaneous application of carcinogenic tar, the incidence of lymphoblastoma and lymphatic leukemia in these mice became as high as 50 per cent, and the disease followed a much more rapid course. The incidence of lymphoblastoma was closely correlated with the carcinogenic potency of the three different tars used. A series of C57 mice treated in the same way failed to show any such lesions. It is concluded that in the presence of a latent predisposition to lymphoblastoma a carcinogenic agent may act as an extrinsic factor leading to the development of this tumor. FROM AUTHORS' SUMMARY

EVALUATION OF THE RISK OF BIOPSY IN SQUAMOUS CARCINOMA. R PATTERSON and J R NUTTALL, *Am J Cancer* **37** 64, 1939

A controlled clinical experiment shows that the incidence of metastases from squamous carcinoma is not increased by biopsy, but no generalization about other tumors can be made from this experiment. FROM AUTHORS' SUMMARY

EFFECTS OF 3,4-BENZPYRENE ON HUMAN SKIN G B COTTINI and G B MAZZONE,  
Am J Cancer **37** 186, 1939

The assumption appears warranted that benzpyrene if applied to human skin for protracted periods would be carcinogenic as it is in animals

FROM AUTHORS' CONCLUSIONS

ACANTHOSIS NIGRICANS AND CANCER OF THE LIVER IN A DOG H O CURTH  
and C A SLANETZ, Am J Cancer **37** 216, 1939

Acanthosis nigricans and carcinoma of the liver in a dog is described Acanthosis nigricans in dogs and human beings is essentially the same process The observation recorded is another instance of the association of acanthosis nigricans and glandular cancer, and is at least consonant with the hypothesis that there is a common genetic factor for acanthosis nigricans and the cancer

FROM AUTHORS' CONCLUSIONS

SUSCEPTIBILITY TO TRANSMITTED LEUKEMIA OCCURRING IN MICE M D SCHWEITZER  
and J FURTH, Am J Cancer **37** 224, 1939

Spontaneous leukemias originating in the highly leukemic stock Ak, in stock Rf, in which the incidence of leukemia is low, and in first generation and other hybrids were inoculated into mice of each of the pure stocks and of various hybrid combinations The leukemias arising in different hybrids behaved in transmission experiments like the Ak leukemias All of these leukemias can be transmitted to almost every member of the leukemic stock Ak and to F<sub>1</sub> generation hybrids, but not to members of stock Rf All hybrid combinations tested have a substantial proportion of susceptible individuals, indicating dominance of inheritance, but further investigation is required to determine if one or two dominant genes are responsible for susceptibility The duration of illness and the anatomic characteristics of leukemia are not modified by the genotype of the host The susceptibility factors of both Ak and Rf leukemias are not allelomorphic Evidence is presented that these genetic factors are specific for susceptibility to transmissible leukemia and differ from those that, according to Loeb, determine susceptibility for normal tissue grafts

FROM AUTHORS' SUMMARY

ABORTIFACIENT ACTION OF SERUM AND URINE FROM CANCEROUS PATIENTS K W  
THOMPSON, T HALE JR and B B WHITCOMB, Am J Cancer **37** 233, 1939

The experiments of Elsasser and Wallace dealing with an abortifacient agent in the serum and urine of patients with cancer have been repeated in part The urine or serum of the majority of the tested cancerous subjects contained a principle which caused termination of early pregnancy in rabbits The principle was not specific for cancer alone, since 3 of 6 apparently noncancerous patients had this agent in the blood or urine, and 1 cancerous subject did not have it in either serum or urine The principal lesions of the animals given injections were apparent in the uteri, where there was degeneration of the fetal structures, including the syncytial and Langhans cells, thrombosis of, and hemorrhage from, the placental vascular structure and, later in the process, an infiltration of the necrotic tissue with leukocytes

FROM AUTHORS' SUMMARY

SEROLOGIC SPECIFICITY OF EXPERIMENTAL TUMORS L DMOCHOWSKI, Am J  
Cancer **37** 252, 1939

The serologic properties of tumors produced in Wistar rats by subcutaneous injections of 0.2 per cent benzpyrene in lard were investigated By intravenous inoculations of rabbits and Wistar rats immune serums were obtained which gave positive complement fixation with heated saline extracts of tumors (benzpyrene tumor, Jensen sarcoma, Walker carcinoma) and, though to a lower degree, with

heated saline extracts of certain normal rat tissues and organs, such as embryo, lung, stomach and intestine. Immune serum against normal rat muscles contains antibodies which fix complement with heated rat tumor and rat embryo extracts even more strongly than with homologous antigen. These cross reactions might be explained by the presence of normal antigens in tumor tissue. Differences in heated saline extracts of normal and tumor rat tissue are discussed in relation to the serologic properties of a dying tissue.

FROM AUTHOR'S SUMMARY

#### HISTOLOGIC CHANGES AND TRANSPLANTATION OF TISSUE SURROUNDING METHYLCHOLANTHRENE PELLETS H. L. STEWART, *Am J Path* **15** 707, 1939

Five per cent methylcholanthrene-cholesterol pellets were placed subcutaneously in C<sub>3</sub>H mice. The purpose of the experiment was to compare the results of histologic examination and of transplantation of the tissue around a carcinogenic agent at weekly intervals during the latent period of tumor development. Tumor-yielding transplants were obtained from pellet mice sacrificed at forty-two and forty-nine days and following a lapse of four weeks at seventy-seven, eighty-two, ninety-one and ninety-eight days. In the sections of the pellet tissue which gave rise to growing tumors when transplanted, atypical cells with characteristics resembling those of malignant cells were present in varying number in the different mice. The results suggest that other factors in addition to the malignant-appearing cells about the hydrocarbon are needed for the development of a tumor on transplantation of pellet tissue into a new host. The results of the transplantation studies indicate that malignant changes may have been induced in cells exposed to the carcinogenic action of methylcholanthrene before the necessary criteria for the histologic recognition of malignancy became fully established.

FROM AUTHOR'S SUMMARY

#### PINEALOMA OF DIFFUSE EPENDYMAL ORIGIN R. P. MACKAY, *Arch Neurol & Psychiat* **42** 892, 1939

Mackay describes a unique tumor of the ependymal lining of all the cerebral ventricles, with obstruction of the sylvian aqueduct and involvement of the tuber cinereum and of the adjacent cerebral tissue, for several millimeters in some locations. The pineal body was not involved but was enveloped by the tumor mass. The tumor was found in a white boy aged 18 who had suffered for three years from confusion, amnesia and delirium and on admission presented the clinical picture of tuberculous meningitis. The tumor consisted of minute round lymphocyte-like cells, which packed the meshes of the reticulin network and were especially numerous around the blood vessels. Other cells were larger, some contained several vesicular nuclei, mitotic figures were common. In addition, ependymal cells (immature and adult) were scattered throughout the tumor, with a tendency to form perivascular palisades. The author holds that the pineal tumor arose from the ependymal lining, though the pineal body itself was not involved. Such a conclusion, in the author's opinion, is permissible in view of the fact that embryologically the pineal body is a differentiated ependymal structure.

GEORGE B. HASSIN

#### VIRUS OF INFECTIOUS MYXOMA R. E. HOFFSTADT and K. S. PILCHER, *J Infect Dis* **64** 208, 1939

The optimal temperatures for the growth of the virus of the infectious myxoma of rabbits on the chorioallantoic membrane is 33-35 C. After repeated serial passage the virus becomes adapted to the embryo as a medium for growth. After it has become thus adapted its titer is not affected by chilling of the embryo. Virus grown on the chorioallantoic membrane produces an infection in the embryo as indicated by gross changes and the blood count and by the fact that the vitality of embryos inoculated with the chick-passed virus is greatly reduced, as indicated by the reduction of the percentage of hatch. As a preservative of the virus of

infectious myxoma, normal rabbit serum is definitely superior to 50 per cent glycerol and physiologic solution of sodium chloride

FROM AUTHORS' CONCLUSIONS

THE ADENOMATOUS GASTRIC LESION IN STRAIN I MICE H B ANDERVONT,  
Pub Health Rep **54** 1851, 1939

The adenomatous lesion of the stomach which occurs spontaneously in practically all adult mice of strain I appears earlier and is more pronounced in the males. Susceptibility to the development of this lesion is inherited as a recessive characteristic, and a number of factors are involved

FROM AUTHOR'S CONCLUSIONS

MELANOMA E K DAWSON, J R M INNES and W F HARVEY, Edinburgh M J  
**46** 695, 1939

The benign melanoma or nevus cell tumor of the skin is a complex malformation, the principal components of which are melanoblasts or melanocytes, these are pigment-forming cells, and in man, in whom melanogenic potency has become restricted ("determined") to epithelium, the common nevus cell tumor is a manifestation of malinduction of the epidermis and its appendages. One form of the nevus cell tumor, the acanthotic nevus, is wholly epidermal. In the human eye and meninges there are found choroidal and meningeal melanotic cell aggregations of the mesodermic pigment cell type. Malignant melanoma in the human being is a carcinoma—epidermal melanocarcinoma in the skin, neuroectodermal carcinoma in the eye. Gaps still exist in knowledge of mesodermal melanoma, and a strict unitary conception of the melanoma may have to be abandoned. The existence of neural melanoma may not be summarily dismissed.

FROM AUTHORS' SUMMARY

PERNICIOUS ANEMIA AND GASTRIC CARCINOMA A W F JENNER, Acta med  
Scandinav **102** 529, 1939

The conclusion is reached that the relative frequency of gastric cancer in association with pernicious anemia is due to the chronic gastritis which is nearly always present with pernicious anemia, also that patients with pernicious anemia should be observed closely for the possible development of gastric cancer. Cancer other than gastric does not have a relatively high incidence in patients with pernicious anemia.

FROM AUTHOR'S SUMMARY

## Technical

IRON HEMATOXYLINS R D LILLIE and W R EARLE, Am J Path **15** 765, 1939

Lillie and Earle show that iron-alum-hematoxylin solutions containing 13 to 17.3 Gm of ferric ammonium sulfate (1.5 to 2 Gm of ferric iron) in a 200 cc quantity stain intensely in five minutes and do not overstain appreciably in thirty minutes. The addition of 15 to 20 Gm of ferrous sulfate (3 to 4 Gm of ferrous iron) preserves the original blue-violet color of the fresh solution for at least three months and preserves satisfactory staining for at least eleven and one-half months. Ferrous ammonium sulfate and ferrous sulfate may be used as sources of ferrous iron. Ferrous sulfate is to be preferred, as it gives a higher concentration of iron for the same weight of salt and is more soluble. It may be generally observed that solutions retaining their blue-violet color give satisfactory staining. Those showing a purplish violet to purplish brown color may also be quite usable, but those showing a yellowish brown color in thin layers are generally inert. Solutions containing 1.5 to 2 Gm of ferric iron in the 200 cc unit quantity to 1 Gm of hematoxylin give adequate nuclear staining in five to seven minutes and do not stain other tissue elements appreciably in thirty minutes. Solutions containing around 0.5 Gm of ferric iron (4.3 Gm of ferric ammonium sulfate) stain

promptly and intensely in four to five minutes and overstain in longer periods. For preservation of the blue non-hematoxylin lake about twice as much of ferrous as of ferric iron is necessary, i. e., quantities of ferrous sulfate or ferrous ammonium sulfate to give 3 to 4 Gm of ferrous iron (15 to 20 Gm of ferrous sulfate). A satisfactory solution which can be kept unchanged for several months is the following:

(A) Ferric ammonium sulfate (violet crystals)	15 Gm
Ferrous sulfate	15 Gm
Distilled water	100 cc
(B) Hematoxylin	1 Gm
Alcohol, 95%	50 cc
Glycerin, chemically pure	50 cc
Mix A and B in equal quantities	

While the foregoing type of solution is remarkably stable and effective, it is felt that greater stability may be attained, and studies now in progress indicate that such solutions can be attained

FROM AUTHORS' SUMMARY

**STOMACH LAVAGE IN THE DIAGNOSIS AND CONTROL OF TREATMENT OF TUBERCULOSIS** A. STADNICHENKO, S. J. COHEN and H. C. SWEENEY, J. A. M. A. **114** 634, 1940

Gastric lavage will find tubercle bacilli that become free in the larger bronchi in a manner not equaled by any other method used at present. Patients with tuberculosis in whom lavage of the stomach does not disclose tubercle bacilli include those with early minimal or moderately advanced lesions which have remained isolated or have been confined to the pulmonary parenchyma or otherwise have not disseminated bacilli into the larger bronchi, those with completely encapsulated lesions, and those with extrapulmonary tuberculosis—types of disease none of which discharge bacilli into the bronchi or pharynx. Completely healed tuberculous lesions and nontuberculous diseases yield uniformly negative results by gastric lavage. As gastric lavage seems to come nearer than any other method to finding all virulent tubercle bacilli, a negative result should be adopted as the ultimate standard for absolute negativity or apparent cure of patients who have had or who have been suspected of having tuberculosis. It is also useful in the elimination of frauds. While gastric lavage is not practical or even necessary for universal use, the efficiency of any method used or recommended for practical use may well be standardized by it. It is an excellent method for controlling the efficiency of collapse therapy. It should be emphasized again that during collapse therapy the absence of tubercle bacilli in only one washing of the stomach does not necessarily signify that the disease is arrested. The washings must be repeated at frequent intervals. Especially is this true when the collapsed lung is re-expanding. No patient with clinical signs of tuberculosis whose sputum is free from bacilli should be considered free from the disease until gastric aspiration has yielded negative results.

FROM AUTHORS' SUMMARY

**ASPIRATION BIOPSY OF THE LIVER** P. IVERSON and K. ROHOLM, Acta med Scandinav **102** 1, 1939

A method is described for aspiration biopsy of the liver. By means of a simple instrument it is practicable to aspirate a tissue column measuring about 2 by 15 mm, sufficient for an ordinary histologic examination. This form of biopsy has now been performed one hundred and sixty times by the authors, it proved unsuccessful in 22.5 per cent of the cases. It causes little inconvenience to the patient, and the risk involved appears to be slight. A tendency toward bleeding is a contraindication. It is possible by this method to demonstrate the presence of acute and chronic inflammatory conditions, tumors, degenerative processes, obstruction to the flow of bile, and other conditions.

FROM AUTHORS' SUMMARY

# Society Transactions

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## NEW YORK PATHOLOGICAL SOCIETY

ALFRED PLAUT, *President*

CHARLES T. OLCOTT, *Secretary*

*Regular Meeting, Dec 28, 1939*

### **Congenital Anomalies of the Coronary Arteries with Cardiac Hypertrophy in Infants** BERYL H. PAIGE

Two cases are reported in which an anomalous origin of the left coronary artery from a pulmonary artery resulted in necrosis, fibrosis and calcification of the left ventricle, hypertrophy of the heart and cardiac failure.

The first patient was a 7 week old Negro girl, with a history of dyspnea. This was first noticed at the age of 2 weeks, thereafter a hacking cough developed. Three days before death the dyspnea became severe and progressively worse, the infant perspired excessively, showed pallor of the mucous membranes and nail beds, and felt cold. The temperature was 98.6 F. Enlargement of the heart was demonstrated by physical, fluoroscopic and roentgen examination. Death occurred on the fourth day after the onset of acute symptoms.

The heart weighed 64 Gm, which is about three times the average normal weight for the age. The right auricle was dilated, and the wall of the right ventricle was hypertrophied, measuring 6 mm in thickness. No gross changes were visible in the myocardium. The left ventricle was dilated, the columnae carneae appeared as a meshwork of branching strands beneath thickened endocardium, and the papillary muscles were atrophied and gray or yellow. The wall of the left ventricle measured from 6 to 8 mm in thickness and showed extensive scars. The right coronary artery originated from its normal site in the aorta and passed to the upper end of the posterior longitudinal sulcus. The left and larger coronary artery arose from the pulmonary artery, behind the posterior pulmonic cusp, and gave rise to the anterior descending, circumflex and posterior descending branches. Microscopically, the myocardium of the left ventricle showed hypertrophy, vacuolation, necrosis and atrophy of the myocardial fibers, as well as fibrosis and calcification.

The second infant, a white pseudohermaphrodite, was admitted to hospital because of a sudden onset of cough, listlessness, pallor and attacks of cyanosis. The temperature was 97.8 F. The alae nasi were dilating, and respirations were rapid and irregular. The heart rate was rapid, a forceful cardiac impulse was felt over the entire chest, and a rhythm suggesting a gallop rhythm was detected. Enlargement of the heart was demonstrated by fluoroscopic and roentgen examination. An electrocardiogram showed right preponderance and inverted T waves, and the deformity of the QRS complexes indicated myocardial damage. The patient improved temporarily in an oxygen tent, but, following a recurrence of dyspnea and cyanosis, died at the age of 2½ months, twelve days after the onset of symptoms.

The heart weighed 64 Gm. The right auricle was dilated, and the wall of the right ventricle was hypertrophied. The left ventricle was dilated, and its endocardial surface was trabeculated, the papillary muscles were atrophied and contained yellow flecks, the myocardium showed extensive scarring. The right coronary artery was normal in its origin but divided into two branches, one of which passed to the left, anterior to the base of the aorta and pulmonary artery, to the



upper end of the anterior longitudinal sulcus. The other branch was small and coursed through the right auriculoventricular groove to the superior end of the posterior longitudinal sulcus. The left coronary artery arose from the right pulmonary artery, just distal to the bifurcation of the main pulmonary trunk. It gave rise to the anterior descending branch and the circumflex branch, which could be traced down the posterior longitudinal sulcus for a distance of 1 cm. Microscopic lesions essentially similar to those found in the first case were present in the myocardium of the left ventricle.

The myocardial injury in such cases is believed to be due to anoxemia of the portions of the myocardium receiving venous blood. Hence the extent of the lesions is dependent on the extent of distribution of the left coronary artery. Consequently, there may be a localized aneurysm of the left ventricle or the diffuse damage of the myocardium demonstrated in the 2 cases presented. Relatively few cases of congenital heart disease have been reported in which an anomalous origin of the left coronary artery from a pulmonary artery is the significant and usually the only cardiac malformation. The condition is not limited to children, in 14 of 21 collected cases the patients were infants, all of whom died during the first year of life.

#### DISCUSSION

ALFRED PLAUT: Was there diffuse fibrosis of the endocardium?

BERYL H. PAIGE: There was fibrosis of the endocardium, with marked thickening.

ALFRED PLAUT: Did the thickened endocardium contain many elastic lamellas?

BERYL H. PAIGE: A stain for elastic lamellas was not used.

ALFRED PLAUT: I am asking this question because there is a group of cases in the large chapter of the so-called idiopathic cardiac hypertrophy of the infant in which diffuse fibrosis of the endocardium seems to be a prominent factor. Among the cases I have seen I did not observe any such malformations of the coronary arteries as were described by you.

BERYL H. PAIGE: I have seen such malformations described in cases in which the endocardium was thickened. Malformations are not limited to such cases as these.

ALFRED PLAUT: May I ask another question, without really expecting an answer? What is your opinion as to the cause of the cardiac hypertrophy?

BERYL H. PAIGE: It has been looked on as diffuse hypertrophy following damage to the left ventricle. Bland and White consider it compensatory hypertrophy of the entire heart.

ALFRED PLAUT: The same condition is found without any anomaly of the coronary arteries.

DR. MUELLER: I should like to ask whether there was any particular pathologic feature in the mothers. Sometimes these pathologic conditions in infants are reflections of maternal conditions.

BERYL H. PAIGE: I was not able to find any evidence of disease or abnormality in the mothers. I went over the histories carefully, and there was nothing to indicate maternal disease or congenital malformations.

#### Acute Hemolytic Anemia and Tubular Nephrosis with Uremia Resulting from the Administration of Sulfapyridine (2-[Paraaminobenzene-Sulfonamido]-Pyridine) J. M. RAVID

A case is reported of fatal acute hemolytic anemia and uremia resulting from administration of sulfapyridine in the treatment of pneumonia in a man 70 years of age. It occurred on the third day of treatment and after a total intake of only 8 Gm. of the drug. The clinical features were those of acute hemolytic anemia, with a sudden drop in hemoglobin to 58 per cent and in the erythrocyte count to 2,700,000, leukocytosis, reticulocytosis, fever, jaundice, oliguria progressing to total anuria, and uremia. The urine contained urobilin, and 10 per cent

of its volume consisted of packed red blood cells and hemoglobin. The blood urea nitrogen was 222 mg, nonprotein nitrogen 258 mg, uric acid 108 mg, cholesterol 310 mg, dextrose 421 mg, and creatinine 88 mg, per hundred cubic centimeters. The icteric index was 30.5, and a direct positive van den Bergh reaction was obtained. The sulfapyridine content of the blood was 11.5 per cent. The patient had complete anuria for the last thirty-six hours of his life. The main anatomic lesions were twofold: first, those secondary to the resulting hemoglobinemia and hemoglobinuria, viz, obstruction of the renal tubules by casts of hemoglobin pigment and its derivatives and hemosiderosis of the liver, kidneys and reticuloendothelial system, and, second, those produced apparently by direct toxic action on the hepatic and renal epithelium. The latter lesion was slight. The lesion in the liver consisted mainly in cytoplasmic vacuolation and accumulation of fat, which was more or less central in distribution. The salient lesion in this case, the renal lesion, which resulted in complete anuria and fatal uremia, consisted in blockage of the tubules with pigment from hemoglobin and its derivatives and, to a lesser degree, in degenerative changes in the tubular epithelium proper.

This blockage was noted mainly in the region of Henle's loops, the recurring limbs and the collecting tubules, the proximal segments of the tubules were dilated. A few calcium deposits in the tubular casts were also noted.

The clinicopathologic picture induced by sulfapyridine is not a syndrome sui generis for this drug alone but appears analogous to those encountered in death following transfusion of incompatible blood (a number of cases), in death following experimental transfusion, in blackwater fever, paroxysmal hemoglobinuria, occasionally in quinine poisoning and in poisoning with certain other chemicals.

The formation of urinary calculi composed of acetyl sulfapyridine crystals, alone or in combination with calcium salts, must also be taken into account in the consideration of the toxic effects of sulfapyridine on the urinary apparatus in general.

Careful daily observations of a patient receiving sulfapyridine with regard to the blood picture and the renal function and the immediate discontinuance of this drug on detection of any deleterious effects on the kidneys or blood should, among other things, be the guiding principle in treatment with sulfapyridine.

#### DISCUSSION

MILTON HELPERN. Some months ago I had occasion to make an autopsy on the body of a young man who had ingested two tubes of a well advertised brand of tooth paste, which has a high content of potassium chlorate. The clinical course was attended by methemoglobinemia, hemoglobinuria and retention of nitrogen in the blood. The kidneys were very similar to those which Dr Ravid described tonight, they were swollen, especially the cortices, and severe degeneration of the convoluted tubules was evident microscopically. The collecting tubules were all distended with hemoglobin casts. The urine found in the bladder at autopsy contained a large number of thick, long, coarsely granular hemoglobin casts.

J. M. RAVID. Dr Helpern's case is a striking one, and the condition appears to belong to the same class of nephropathies which I have described, namely, chemical obstructive and degenerative nephrosis.

#### Visceral and Vascular Lesions in Scleroderma ABRAHAM D. POLLACK

The association of visceral and vascular lesions with scleroderma suggests that scleroderma may in certain instances represent merely a local manifestation of a disease state in which widespread alterations can be found in many other organs and tissues of the body.

Two cases of scleroderma showing certain similarities in clinical course are presented. Preceding the full development of the sclerodermatous change, each patient experienced premonitory circulatory disturbances in the extremities. Both had arthropathy, evidence of active renal disease, anemia, leukocytosis and fever.

Anatomically, the most striking change is a fibrinoid deposit in the walls of small arteries and arterioles in many organs. In the first case the fibrinoid deposits were limited almost exclusively to the intima. In the second, the fibrinoid degeneration was found throughout the vessel wall. The intimal proliferation and periarterial inflammation recall the vascular lesions seen in periarteritis nodosa. The vascular lesion is most evident in the kidneys. In the second case the kidneys showed, in addition to the vascular changes, a peculiar type of glomerulonephritis, with the so-called "wire loop" changes, as seen in disseminated lupus erythematosus.

The genesis of the vascular and mesenchymal alterations in both cases is, at the present time, unclear. It is apparent, however, that certain forms of scleroderma must be considered together with that puzzling group of conditions which have been called "toxic" or "allergic" diseases. These include disseminated lupus erythematosus, dermatomyositis and periarteritis nodosa.

#### DISCUSSION

GEORGE BAEHR. Clinically, scleroderma represents a degenerative and atrophic lesion in the skin, it is a symptom of disease and not a disease entity. It occurs most commonly in persons who have a sympathetic vasomotor disturbance, so-called Raynaud's disease. As a result of long-standing Raynaud's disease sclerodermatous changes occur in the skin of the extremities, face and other parts of the body. Similar localized disturbances occur in the skin of the extremities secondary to various forms of joint disease of the hands and feet.

The case of diffuse scleroderma with visceral and vascular lesions which Masugi described seems to be identical with the cases which Dr. Pollack has presented and with the case which Talbot, of Boston, described last year. It seems to me that these 4 cases do constitute a disease entity. In making this statement, I should emphasize again that this is only one form of diffuse scleroderma. I think it would be wrong to give the impression that all cases of diffuse scleroderma necessarily belong in this group, for scleroderma in itself is merely a superficial manifestation possibly of a variety of different conditions. Similarly, an erythema on the bridge of the nose spreading in a butterfly fashion on the malar eminences of the face does not in itself warrant a diagnosis of lupus erythematosus in the absence of the complete clinical picture. An erythema of this type and in this location can be due to other causes.

In the 2 cases which Dr. Pollack described, and those of Masugi and Talbot, one has a common group of clinical and pathologic phenomena—in other words, a distinctive clinical picture and pathologic process. There are a long, intermittently febrile course, progressive changes in the skin characteristic of scleroderma, urinary evidences of changes in the blood vessels of the internal organs and a tendency toward involvement of joints and serous membranes.

I had the opportunity to study both patients clinically, the man six months before scleroderma developed. The persistent presence of red blood cells in the urine indicated to me, even at the first observation of the patient, that there must be some vascular lesion in the viscera. The tendency to swelling of the hands and feet and the peculiar glove-like cyanosis of the hands I felt obliged to ascribe to vascular phenomena resulting in stasis in capillary circulation, perhaps most significant on the venous side of the capillary bed. Only after many months did this obscure vascular disturbance result in almost universal scleroderma. In addition to the clinical evidence of vascular disease in the kidneys, these patients had involvement of the synovial membranes of the joints and of the serous membranes of the pericardium, pleura and peritoneum. The first patient had conspicuous leukopenia at one time and a tendency toward depression in the hemoglobin and red cell count. In other words, the clinical picture, as well as the pathologic process in the vessels and serous membranes, bears a close resemblance to the clinical phenomena and the lesions seen in lupus erythematosus, and yet the cutaneous manifestations are so different.

Concerning the cause of the condition, I think it is best to confess complete ignorance. It seems to me to be unwise to label the condition allergy or to use any other obscure term, for this would merely serve to hide the fact that one has not the ghost of an idea what the essential nature of the process may be. It is important now in considering all cases of diffuse scleroderma that one be aware of the fact that in some of them the condition may represent a diffuse systemic disease affecting not only the skin but vascular structures throughout the body, as well as the serous membranes and the synovial membranes, and that it may fit into a disease entity having some relationship perhaps to diseases like lupus erythematosus and dermatomyositis.

J M RAVID I should like to ask Dr Pollack concerning the course of the blood pressure in the first case and the course of the renal function in both cases during the progress of the disease.

ABOU D POLLACK Both patients had normal blood pressure throughout their illnesses until the terminal event, when the blood pressure rose to a high level only just before death. In the first case no special studies of the blood were made in relation to renal function, in the second case there was terminal retention of nitrogen.

#### Certain Diseases Observed in North China C H HU (by invitation)

This was a brief presentation, with over 80 photographic illustrations, of certain diseases observed in the Peiping Union Medical College, Peiping, China. The diseases included follicular hyperkeratosis due to avitaminosis A, nutritional edema, typhoid fever, bacillary dysentery, leprosy, tuberculosis, syphilis, relapsing fever, typhus fever, kala-azar, amebiasis, schistosomiasis, cysticercosis, echinococcus infection, Laennec's cirrhosis, thromboangitis obliterans, and various kinds of tumors.



ALFRED PLAUT, *President*

CHARLES T OLCOTT, *Secretary*

*Anniversary Meeting, Jan 25, 1940*

#### Thromboarteritis of the Pulmonary Artery with Chronic Obstruction in the Pulmonary Circulation B M VANCE

A white man 52 years old fell downstairs, sustaining a laceration of the scalp in the right parietal region, after which he was unconscious for several minutes. Several hours later he became markedly dyspneic and cyanotic. He died after six days, with evident signs of right-sided cardiac failure.

The necropsy disclosed a muscular, slightly obese white man with a laceration of the scalp as noted, but no other sign of injury. The skull and brain were normal. The right ventricle and auricle of the heart were markedly distended and hypertrophic, while the left ventricle and auricle were small in comparison. The aorta, coronary arteries, cerebral arteries and femoral arteries and veins were normal aside from slight intimal sclerosis. The lungs were well aerated anteriorly, but posteriorly they showed a few depressed areas of atelectasis. The air passages were normal. The main stem of the pulmonary artery and its right and left branches were occluded with a fragile, grayish red thrombus. The larger branches of the intrapulmonary arteries were filled with a firm, layered thrombus, while the arteries 2 to 6 mm in diameter had thick fibrous walls and contained recent thrombi here and there. The pulmonary veins were normal. Other conditions found at necropsy were passive congestion of the liver and spleen, slight subcutaneous edema around the ankles, moderate arteriosclerotic changes in the kidneys, cholelithiasis, slight prostatic hypertrophy and an old false joint in the shaft of the left ulna.

Microscopic examination of the pulmonary arteries disclosed that the vessels of 2 to 6 mm caliber were narrowed or occluded by marked proliferation of the subendothelial connective tissue, which was extensively canalized by minute distended blood vessels. In some arteries the canalized tissue filled the lumen, but in others it narrowed the lumen and pushed it to an eccentric position. The elastic layer of these arteries showed widespread splitting. The larger vessels contained organizing thrombi, with polymorphonuclear leukocytes grouped in the vessel wall around the thrombotic area. In some regions the large trunks of the pulmonary artery were edematous because of fluid in the tissue spaces. In other areas the vasa vasorum were distended and surrounded by numerous lymphocytes. The smallest pulmonary arteries were normal except for a few vessels which showed cellular proliferation of the subendothelial tissue and narrowing of the lumen. There was no atheromatous degeneration or calcification in any of the pulmonary vessels.

The principal lesion in this case was nonspecific arteritis of the pulmonary artery associated with thrombus formation, which caused gradual narrowing of the lumen of the artery and chronic obstruction in the pulmonary circulation. The etiologic factor in this case could not be determined, but the process had apparently started in the medium-sized arteries of the lungs and from there extended to the larger trunks.

#### DISCUSSION

ANDREA SACCONI I should like to ask if the vessels of the other organs, the spleen and the kidney, were investigated, because many times this type of pathologic picture in the lung is associated with proliferative endarteritis in the other organs.

ALFRED PLAUT What was the profession of the patient?

B M VANCE He was a janitor. What his profession was before that I was not able to ascertain.

ALFRED PLAUT I had a special reason for asking that question. In going over the literature of pulmonary thromboarteritis I found some relative predominance of the condition in gardeners and in people who might have been exposed to lead.

In 1935 at the Medical Fortnight I exhibited a case of pulmonary endarteritis which differed from the case which Dr. Vance presented in that the disease attacked the smaller vessels.

B M VANCE The other arteries of the body showed a slight amount of endarterial change, a thickening of the intima of the usual arteriosclerotic type, which was not particularly pronounced. There was slight phlebosclerosis of the right femoral vein. The bronchial artery on section exhibited definite narrowing of its lumen due to arteritis obliterans. The aorta was normal except for slight intimal sclerosis. The arteries in the other organs of the body were normal.

#### Contralateral Adrenal Atrophy Associated with Cortical Adrenal Neoplasms TOBIAS WEINBERG

The purpose of the presentation of the 3 cases of adrenal cortical neoplasm is to reemphasize the frequent occurrence of contralateral adrenal atrophy in association with such a neoplasm. All 3 patients were women, and all presented the Cushing syndrome. Two were operated on and found to have cortical adenoma. Both died postoperatively. The third was found to have carcinoma of the adrenal cortex with metastases in the liver and lungs. In all 3 cases the contralateral adrenal was definitely atrophic. A review of the literature for the past fourteen years yielded 34 cases of adrenal cortical neoplasm in which the clinical diagnosis of the state of the adrenals was confirmed by autopsy. In 21 of these cases, or approximately 62 per cent of the total number, the contralateral adrenal showed atrophy.

It is suggested that in order to prevent the high mortality incident to operative intervention, desoxycorticosterone pellets, for example, be implanted at an adequate time before operation so that they may have effect, also that once the

diagnosis of an adrenal cortical neoplasm is made, operation should not be delayed, so that functional inactivation, if not actual atrophy of the contralateral adrenal, may be avoided

**Influence of Sulfanilamide and Sulfapyridine on the Evolution of Experimentally Induced Pneumococcic Pneumonia in Rats** DAVID GOLDSTEIN  
(by invitation) and IRVING GRAEF

This paper will be published in a later issue of the ARCHIVES

DISCUSSION

REUBEN OTTENBERG Were pneumococci found in the abscesses in the treated animals, or were they sterile abscesses?

S C BUKANTZ (by invitation) From the charts it appeared that the first negative cultures from the lungs of treated animals were encountered on the fifteenth to eighteenth days. Later on it was brought out that bacteria were not present in the lungs of the treated animals twenty-four hours after treatment. Is there a difference between spread and culture? I wondered whether any studies of the concentration of the drugs in the blood were done to give some idea of what levels were reached in the respective groups of treated animals, and whether the treatment was ever varied so as to begin at intervals longer than two hours after inoculation of the pneumococci.

DAVID GOLDSTEIN In reply to Dr Ottenberg's question, there were several abscesses in fifteen day animals which failed to reveal pneumococci on careful study both by stains and culture. However, some of the abscesses in the treated animals put to death at four and five days revealed the presence of pneumococci.

A question was raised about confusion in the reports of the negative cultures. I think that can be clarified by considering again the mode of presentation of the results in the charts. Only positive cultures were recorded against the total number of animals for each day. Thus, at one day there were 12 sulfanilamide-treated animals, 5 of which were shown to have positive lung cultures. As time went on, the incidence of positive lung cultures grew less frequent. On the second day there were 3 positive cultures for 8 animals, on the fourth day 2 for 9 animals, and for the 8 animals of the fifteenth day there were no positive cultures. Sulfapyridine yielded similar results.

S C BUKANTZ Then that is the first time when all animals in a group gave negative lung cultures?

DAVID GOLDSTEIN Yes. The fifteenth day was the first time that all cultures were sterile. In reply to your other question, we determined the concentration of the drug in the blood in our experiments with sulfapyridine. We made no determinations in the experiments with sulfanilamide but used Marshall's data on rats as a standard, from which it was possible to estimate the level of sulfanilamide to be 15 mg per hundred cubic centimeters. The level of sulfapyridine in 4 rats was determined to be approximately 20 mg per hundred cubic centimeters. It varied between 14 and 26 mg per hundred cubic centimeters.

S C BUKANTZ What length of time elapsed between the administration of sulfapyridine and the determination of the concentration of the drug in the blood?

DAVID GOLDSTEIN One estimation was made four hours after the administration of the drug, and that was 14 mg per hundred cubic centimeters. With the same dose of 200 mg daily there apparently was a cumulative effect, so that the concentration rose to 26 mg per hundred cubic centimeters on the third day.

We have a small series of 8 rats in which delayed treatment was studied. These were thought too few for presentation. Half of this group survived, and half died. They were all critically sick, and those which died, died shortly after the first treatment. The results in those which survived and were put to death on the tenth day roughly paralleled the results in the treated rats presented this evening.

**Aplastic Anemia** CORNELIUS P RHOADS (by invitation)

A clinical and pathologic study of anemias refractory to treatment with accepted hemopoietic agents is reported

The histologic changes of the bone marrow are described, and the changes present in the idiopathic disease are compared with those found in clinically similar conditions of known toxic nature

The natural history and the symptom complex of the disease are discussed as observed in the 66 cases included in this report, and particular reference to remissions is made

Studies of the metabolism of bile pigments in patients with refractory anemia are reported, and the results compared with the results in similar studies of normal persons

The excretion of porphyrins in refractory anemia and in other disorders of the blood is discussed, since evidence recently presented by Dobriner shows that the rate of excretion of coproporphyrin type I may be used as an index of the rate of cell formation by the marrow and that type III is a pathologic product

The results of tests of liver function in 21 of the cases in the series are presented and discussed

The results of test of ability to conjugate cyclic compounds into sulfates and glucuronates are reported and the significance of these results considered

The relationship of refractory anemia to leukemia is mentioned

Finally, certain considerations of causes and of treatment are discussed, and the histologic changes in idiopathic refractory anemia compared with those of refractory anemia of known toxic cause in animals

## DISCUSSION

CHARLES L FOX JR Dr Rhoads mentioned Dr Rimington's and Dr Brownlee's work on porphyrins in the urine of animals treated with sulfanilamide and various antipyretic drugs They showed that methemoglobinemia paralleled the excretion of the type III porphyrin Do your studies show such a parallelism?

CORNELIUS P RHOADS No, my associates and I have never found consistent methemoglobinemia in these patients There is a hitch to the work of Rimington and Brownlee, since their work was done principally on rats They showed that rats had an increased output of type III porphyrin when poisoned by certain compounds with aromatic amines We are not at all sure that normal rats do not excrete type III porphyrin instead of type I as does man We have shown that type III porphyrin is excreted by normal rabbits, and work on rats is in progress

## CHICAGO PATHOLOGICAL SOCIETY

S A LEVINSON, *President*

EDWIN F HIRSCH, *Secretary*

*Regular Monthly Meeting, Jan 8, 1940*

**Radiosensitivity of Primary Carcinoma of the Lung and the Effect of Irradiation on Time of Survival** PAUL E STEINER

Twenty-one primary carcinomas of the lung which had been treated with known amounts of roentgen rays were studied post mortem Marked degenerative changes were observed in some, but in none was the therapy lethal for all of the tumor cells, although the therapeutic dose was as much as 5,000 roentgen treatment than adenocarcinoma and squamous cell carcinoma Marked gens Contrary to the anticipated result, undifferentiated cell carcinoma appeared to be more radioresistant or to have a higher degree of recuperability following

cell degeneration was seen in 2 squamous cell carcinomas treated with 1,670 r and 1,490 r, respectively, although in others larger doses produced no visible effects. The carcinomacidal dose for primary cancer of the lung appears to be over 5,000 r. In tissue from a small frontal bone metastasis of an undifferentiated cell type of pulmonary cancer 3,800 r had destroyed all tumor cells. The tissues of the lung, pleura, pericardium, heart and esophagus adjacent to the tumors had no recognized damage from the irradiation. The histologic effects of irradiation on the tumor cells resembled those described by others for other types of tumor. The average time of survival from the date of the first treatment in five patients whose cancers were given over 3,000 r was one hundred and thirty-one days. Life was not notably prolonged in the group whose cancers were irradiated when compared with a group of 53 whose cancers were not irradiated.

This paper will appear, with details of histologic changes, the technic of therapy and illustrations, in the *Archives of Internal Medicine*.

#### DISCUSSION

P J MELNICK. It is encouraging to note an increasing interest in the study of tissue changes induced by radiant energy. The histologic changes that I have observed in human and animal tumors are the same as those demonstrated, and my understanding of them is the same. I shall emphasize two features. The rate of delivery of radiant energy is significant, i. e., high intensity over a short period in contrast with low intensity over a longer time. Large doses in a short time cause necrosis, fractional doses cause retrogressive changes in the cells, due to cumulative effects. Also, certain tumor tissues show the full cumulative effect, but the small cells, according to Dr Steiner's report, do not. The reason is not known.

H C SWEANY. Is there any evidence that small doses stimulate growth of the tumor? How were the biopsy tissues taken?

I PILOT. Did roentgen films demonstrate shrinkage of the tumors during treatment?

S R ROSENTHAL. Certain bronchial tumors contain anaplastic tissues, occasionally tubules, and are termed adenoma.

S A LEVINSON. Was there a difference in the incidence of metastases as between irradiation and nonirradiation?

PAUL E STEINER. Nothing was observed to suggest that small doses of radiant energy accentuated the growth of the tumors. Some biopsy tissues were obtained through the bronchoscope, other tissues for control were metastases not subjected to radiation. There were 2 "adenomas" in the material studied. They were comparatively benign but spread by continuous slow growth. Graham has spoken of them as mixed tumors. There was no difference in the development of metastases in the treated and untreated groups.

#### Effect of Colchicine on Human Tissues W O BROWN

Colchicine produces a diminution in the size of experimental tumors, as well as nuclear changes in the cells of almost all parenchymatous organs. The effects of colchicine are most prominent in cells undergoing mitosis, which may be arrested for a variable time. Bizarre nuclear configurations result, and not infrequently cell death. Three patients with inoperable malignant growths died in the course of colchicine treatment. The results of the autopsies indicate that the action of colchicine is not selective but that its effects are most marked on those cells having a high rate of metabolism and mitosis. There is nothing specific about the morphologic appearance of the cells arrested by colchicine. Identical changes are frequently found in routine autopsy material, without reference to the cause of death. Owing to the depressing effect on bone marrow, agranulocytosis and aplastic anemia occur frequently. An observation not recorded



previously is the production of peripheral neuritis with fatty degeneration of the myelin sheaths

The complete report will be published elsewhere

#### DISCUSSION

PAUL E. STEINER Is there enough evidence in patients or experimentally in animals to justify continued use of the drug?

A. B. RAGINS In my studies of tumor tissues with colchicine therapy, the growth of cells seemed to have been halted in the metaphase

F. QUEEN Were these patients killed by the colchicine therapy?

W. O. BROWN There are only a few experimental studies on the effects of colchicine in animals. Dustin in 1908 noted that larger doses caused aplastic anemia and that small doses stimulated the bone marrow. Apparently the arrest of tumor tissue growth is in the metaphase. The nuclear changes later lead to the conditions observed. In 2 patients death resulted from agranulocytosis caused by the colchicine, although both patients had extensive carcinoma

#### Tetralogy of Eisenmenger M. L. V.

The types of congenital abnormality of the heart that permit survival to adult life are relatively few. Among these are the tetralogy of Fallot and the tetralogy of Eisenmenger. The tetralogy of Fallot consists in a dextroposition of the aorta, a defect of the interventricular septum, right ventricular hypertrophy and stenosis of the pulmonary tract. The tetralogy of Eisenmenger differs from that of Fallot in not including pulmonary stenosis. Instead, the pulmonary artery is either normal in size or, more often, is dilated. A 19 year old youth was thought clinically to have the tetralogy of Fallot with mitral stenosis. At autopsy the tetralogy of Eisenmenger was found with stenosis of the mitral orifice. Both the aorta and the pulmonary artery emerged from the right ventricle. The only outlet of the left ventricle was a defect of the ventricular septum in the region of the pars membranacea. The pulmonary artery was wider than the aorta. The outlet regions of the arterial trunks were separated by an arch of musculature at the base of the right ventricle. In addition there were coarctation of the aorta, a patent ductus arteriosus, an abnormally formed left auricular appendage and marked mitral stenosis. From the standpoint of transposition, this anomaly is type II, according to Spitzer, or a partial transposition, according to Rokitsansky. The embryologic explanation of this anomaly is part of the general explanation of transposition which will be discussed in another communication. The tetralogy of Eisenmenger may be classified according to the degree of transposition into (a) transposition with an aneurysm of the membranous septum (the case of Libman and Abbott belongs here), (b) a riding aorta with a defect of the interventricular septum (this includes most of the cases recorded in the literature) and (c) partial transposition, in which the aorta and pulmonary artery arise from the right ventricle. This case apparently is the only one of the last variety to be recorded in the literature. It also seems to be the only one on record complicated by stenosis of the mitral orifice, the result of an old acquired endocarditis.

#### DISCUSSION

O. SAPHIR The anomaly which Eisenmenger described was regarded at first as the tetralogy of Fallot. Appreciation of these anomalies is not so difficult if one remembers that the disturbance is due to torsion defects of the bulbus.

#### Acute Necrosis of the Adrenal Glands with Tryparsamide Therapy M. G. BOHRD

Wells, Humphreys and Work reported selective necrosis of the adrenal glands in a woman who received germanin (Bayer 205) for pemphigus. They suggested that the increase in Addison's disease due to cortical atrophy may be the result of toxic destruction by some drug or drugs as yet undisclosed. A man aged 51

years received twelve injections of tryparsamide for dementia paralytica. He died within a half hour after the twelfth injection, apparently in shock. Necropsy showed, in addition to the lesions of dementia paralytica and syphilitic aortitis, marked swelling and hemorrhagic necrosis of both adrenal glands. The two weighed 50 Gm. Histologically, both cortex and medulla were necrotic, but small islands of cortical and medullary cells were apparently intact. No other cause for the adrenal necrosis was found, and it seems that the tryparsamide injected may have been the cause. Attempts to produce adrenal necrosis in animals by injections of tryparsamide have failed. Germanin and tryparsamide have no obvious chemical similarity. Both drugs are effective in the treatment of trypanosomiasis. This suggests a pharmacologic similarity, because the only 2 cases in which a drug may have been the cause of necrosis of the adrenal glands had this similarity of therapeutic action. While this report strengthens the conception of Wells and his associates that drugs may be responsible for the increase of cortical atrophy of the adrenals, there is the possibility that acute infections, principally meningococcal infections, may be associated with necrosis of these glands. In a case in which the condition accompanied lobar pneumonia, thrombosis was present in one gland but not in the other, suggesting that thrombosis of the adrenal vein, often claimed as the cause of adrenal necrosis, may be secondary to the necrosis.

## DISCUSSION

E. M. HUMPHREYS. This report adds another drug to the small number which seem to have some relation to selective necrosis of the cortex of the adrenal gland. Since our report (*J. A. M. A.* **109**:490, 1937) 2 more cases in which treatment with germanin (Bayer 205) was followed by necrosis of the adrenal cortex have been published (Tomlinson reported one of these [*Arch. Dermat. & Syph.* **38**:555, 1938]). Some experimental studies have demonstrated that germanin causes selective necrosis of the cortex without affecting the cells of the medulla.

PAUL R. CANNON. I have recently observed focal necrosis in the cortex of both adrenal glands with phenobarbital poisoning and subsequent agranulocytosis.

S. R. ROSENTHAL. Sulzberger observed varying results with arsphenamine in animals, results which he was unable to explain. Perhaps some dietary condition is a determining factor.

O. SAPHIR. There are two different diseases in the adrenal glands: (1) hemorrhage with necrosis of both cortex and medulla and thrombosis of the veins and (2) selective necrosis of the cortex without injury of the medulla. Atrophy of the cortex with focal regeneration probably does not occur after diffuse necrosis.

M. G. BOHRD. I do not think that a causal relation between the drug and the lesions has been established in this case. Nor does thrombosis explain all of the changes.

## PATHOLOGICAL SOCIETY OF PHILADELPHIA

J. H. CLARK, *President*

H. L. RATCLIFFE, *Secretary*

*Regular Meeting, Jan. 11, 1940*

### Larvae of *Multiceps Serialis* in a Gelada Baboon. Possible Relation to Hydatid Disease. H. L. RATCLIFFE

*Multiceps serialis* is one of a group of cestodes which utilize various herbivorous mammals as intermediate hosts and complete their development in the intestines of dogs. Because the larvae attain considerable size, the larval stages of these tape-

worms are much better known than their maturity. Certainly the best known of these larvae is the hydatid cyst of *Echinococcus granulosus*.

Occasionally, instead of growing as a single cyst, *Echinococcus* assumes a multilocular form which lacks a limiting capsule and infiltrates surrounding tissues in a manner suggestive of a malignant tumor. Both forms of cyst localize most frequently in internal organs but at times may involve skeletal muscles or subcutaneous tissues.

The larva of *Multiceps serialis* also develops as a multilocular cystic mass that infiltrates and destroys adjacent tissues, a feature well illustrated in the present case.

An adult male baboon, *Theropithecus gelada*, had been exhibited at the Philadelphia Zoological Garden for about five years. This animal, as is characteristic of the species, had a thick mass of long hair covering the neck and shoulders, so that a subcutaneous mass would not be seen until large enough to displace this hair, unless the animal was restrained for examination. The presence of the parasite was not suspected until the overlying skin had ulcerated. The animal died of circulatory collapse about twelve hours after he had been anesthetized for examination. The large multilocular cystic mass which had developed in the subcutaneous tissues and superficial muscles of the shoulder and arm corresponded equally well to descriptions of *Echinococcus multilocularis* and *Multiceps serialis*. However, examination of scolices from the cyst showed that it was an example of the latter organism. Differentiation of the two parasites, while entirely of academic interest, must depend on the morphologic appearance of scolices from the cysts, since it is not possible at present to distinguish them by serologic tests.

#### Neuropathologic Study of a Case of Tetanus W. P. JENNINGS and R. P. CUSTER

A young white woman was admitted to the surgical service of Dr. John C. Howell at the Presbyterian Hospital with tetanus, which had developed sixteen days after lodgment of a wooden splinter in the left knee. Tetanus antitoxin was given prophylactically, but the splinter was not located until autopsy, 84,000 units of serum was given therapeutically. A serum reaction occurred, and, following further administration of the serum, shock developed, this subsided, but the woman died soon thereafter, on the day following admission, in an exacerbation of the disease.

Postmortem examination disclosed the foreign body in the soft tissues of the left knee, localized by proliferating fibrous tissue. Tetanus bacilli were recovered from pus in the wound. There was marked degeneration of all of the organs, with extensive hemolysis. The brain was tightly applied in the cranial cavity, the tissue was softened, the spinal fluid was increased, and there was marked cerebellar coning. The meninges appeared normal. Cut surfaces were markedly engorged, and there was staining of the tissues with blood perivascularly. Spinal cord tissue, particularly the cervical and thoracic segments, appeared edematous and congested. The lesions of the nervous system were predominantly degenerative. There was degeneration of the third and fourth cerebral cortical layers, with accompanying gliosis and widespread vascular endothelial proliferation. The ganglion cells were undergoing chromatolysis. Tract demyelination, particularly in the region of the fourth ventricle, was noted. Cerebellar granular and molecular layers were degenerated, and the Purkinje cells were undergoing extensive chromatolysis. A section of the sciatic nerve underlying the site of infection showed extensive loss of fibers, demyelination and perineural proliferation. A corresponding section of the right sciatic nerve was edematous, and there was demyelination with sheath proliferation of lesser extent. The process involving the right sciatic nerve was interpreted as predominantly an acute degenerative process, in contrast to a more chronic process with reactive phenomena in the sciatic nerve on the involved side. Finally, all the tissues showed evidence of hemolysis.

The papers of Abel may be recalled in which he favored a peripheral and a central action of the tetanus toxin and expressed the belief that action on the

nervous system resulted through blood stream-lymphatic distribution rather than by direct transmission by nerve or by perineural lymphatics to the meninges. Experimentally, lethal doses injected intravenously were apparently fixed in all of the tissues in an irrecoverable form, any toxin in excess of the lethal dose could be recovered from the circulation.

We interpret our findings as support for the experimental evidence of Abel that the toxin is lymph borne and blood borne and that there is general toxemia, for not only were degenerative changes for the most part rather uniform and generalized throughout the central nervous system but all of the other tissues examined shared equally in the degenerative process. The second reason is that the first symptom apparently was general malaise, followed by dysphagia, and soon thereafter by generalized involvement, if the toxin had traveled along the nerve, the affected segment of the spinal cord should, supposedly, have given prominent localizing signs early. The assumption of nerve transmission cannot be justified on the basis of the sections. Since more proximal segments of the nerves were not examined, no knowledge is at hand as to whether they were similarly involved to the same relative extent. Examination of the spinal cord itself showed symmetric degeneration.

### **Pulmonary Pneumatocele (Bullous Emphysema, Giant Bullae of the Lung, Polycystic Lung) Report of a Case** ESMOND R LONG

A Negro approximately 40 years old entered the outpatient department of the Philadelphia General Hospital in acute respiratory distress, wheezing and gasping for breath. He died ten minutes after entrance. The available past history was meager. He had served a number of prison sentences for violation of the narcotic laws, and had once been examined in the outpatient department of another hospital. In each of these institutions he gave a history of asthma and repeated colds. No roentgen films were made during life, and no definite diagnosis was made.

Autopsy disclosed multiple pneumatocele of the lungs. Each upper lobe was composed largely of giant bullae, which compressed the functional portion of the lung below. Acute terminal dilatation of these bullae, dependent on a valvelike opening to each, was apparently the cause of death.

Multiple pneumatocele of the lung is not rare, but this occurrence of it seems to merit record because of the huge size of the air sacs, which reached diameters of 12 to 15 cm in each upper lobe, and because of the acute termination. The causes in most cases are obscure, and the designations of the condition are confusing. In many instances it has been called congenital cystic disease of the lungs, but in most cases it is not congenital, and the air sacs are not cysts according to the usual definition of a cyst. The multiple pneumatocele under consideration appeared to be the sequel of old diffuse chronic infection and resultant fibrosis of the bronchiolar walls.

### **Genesis of Polycystic Disease of the Kidneys** ROBERT F NORRIS and LEON HERMAN

The important theories of the origin of polycystic disease of the kidneys are reviewed. Congenital polycystic kidneys are described as observed in 4 patients. From serial sections, several collecting ducts and cysts in the kidneys of each patient have been reconstructed to scale in drawings. Evidence is given that for a long period in fetal life the development of these kidneys was normal. After differentiation of the metanephrogenic anlage and after the union of its elements with collecting ducts, there occurred focal cystic dilatations of uriniferous tubules and collecting ducts followed by isolation of the dilatations as cysts of segments of these nephrons. Continued proliferation of these elements and rupture of their walls resulted in anastomoses among these cysts. The changes are thought to be degenerative since they resemble the stages in normal degeneration of the mesonephros and of the normal vestigial elements of the metanephros. In the case of

polycystic kidneys, therefore, to a variable degree the metanephros is abnormally provisional. Such an explanation appears to be applicable to most of the congenital anomalies associated with polycystic kidneys and to be compatible with the hereditary nature of the disease. The fundamental cause of polycystic disease of the kidneys must be in the germ plasm.

#### A Scientific Unit for Recording Erythrocyte Sedimentation Rates J W CUTLER

Lack of a universal technic has been a serious stumbling block to more widespread adoption of the blood sedimentation test in general practice. In every one of the technics described in the literature the unit of comparison (usually the drop at the end of one hour expressed in millimeters), while it reflects the rapidity of sedimentation in a general way, fails to give an accurate idea of the rate of settling. Furthermore, it also reflects anemia in the packing of the cells in the bottom of the tube within the first hour in rapidly settling blood.

The object of the present technic is to determine the rate of settling of the rouleaux at a time when the rate is least influenced by the period of aggregation of the red cells into rouleaux, on the one hand, or their packing in the bottom of the tube, on the other. The essential features are as follows. Cutler sedimentation tubes of 1 cc capacity are used, which are graduated into 50 mm divisions, with 0 at the 1 cc level. One tenth of 1 cc of a 3.8 per cent sodium citrated solution and 0.9 cc of blood obtained by puncture of a suitable vein are gently mixed in a 2 cc syringe and poured into the sedimentation tube, and the tube is placed in a special rack. The position of the sedimenting column of erythrocytes is determined every five minutes for one-half hour and recorded on special charts.

The biggest drop in any five minute period during the first half hour is the maximum sedimentation rate in five minutes (MSR) that the rouleaux will attain in the Cutler tube, no matter how many readings are taken. This maximum rate becomes the unit of comparison.

A maximum settling of 1 mm or less in five minutes during the first half hour is normal. Anything more than this is abnormal and therefore pathologic. A maximum rate of 1.5 to 3.5 mm, although pathologic, is usually not associated with constitutional manifestations of disease such as a fever or a rapid pulse. In such instances the pathologic process responsible for the increased rate is usually latent or subclinical. A maximum sedimentation rate of 4 to 9.5 mm in five minutes, on the other hand, is usually associated with constitutional symptoms, but these are of mild to moderate severity. A maximum rate of 10 mm or more in five minutes is almost invariably associated with marked constitutional symptoms.

This is all there is to a scientific determination and interpretation of the sedimentation rate. This unit—maximum sedimentation rate in five minutes (MSR)—needs no correction for anemia. It cannot be misunderstood, for it means just what it says. It is important to remember that the "MSR unit" is different in tubes of different length.

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J H CLARK, *President*

H L RATCLIFFE, *Secretary*

*Regular Meeting, Feb 8, 1940*

#### Nature, Incidence and Duration of Hodgkin's Disease E B KRUMBHAAR

The substance of this address can be found in the author's paper "A Symposium on the Blood and Blood-Forming Organs," published by the University of Wisconsin Press, Madison, Wis., 1939.

**Idiopathic Hypertrophy of the Heart in Infancy** IRVING J. WOLMAN

The cause of idiopathic cardiac hypertrophy is not known. Though rare at all ages, the disorder is encountered most frequently in infancy. Clinical and pathologic data on 5 patients are presented, these were infants aged between 4 and 18 months, who had died of sudden, unexpected circulatory failure. In none had the presence of heart disease been suspected during life. The hearts were dilated but were free from anomalies and from evidences of rheumatism and diphtheritic intoxication. The musculature was diffusely hypertrophied, each heart weighed from two to three times more than normal. In 4 of the 5 specimens wandering cells, chiefly mononuclears, were discovered within the interstitial connective tissue. The cause of the cardiac changes was not apparent, stains for glycogen showed no excess. Aerobic and anaerobic cultures of the ventricular muscle of 3 of the hearts were negative. The infants had not been anemic or syphilitic. It is possible that some obscure nutritional deficiency lies at the basis of this puzzling disorder. A review of the histories of the feeding of these infants shows that 4 of them had been receiving inadequate quantities of vitamin D in their daily diets, 2 had florid rickets at the time of necropsy. No other clues as to the causes were obtained.

## Book Reviews

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**Architecture of the Kidney in Chronic Bright's Disease** Jean Oliver, Professor of Pathology, Long Island College of Medicine, Brooklyn Cloth Pp 257, with 112 illustrations Price \$10 New York and London Paul B Hoeber, Inc, 1939

This impressive volume comes at an opportune time, for it brings refreshment of mind and valuable information to pathologists who have long been exasperated by the flow of petty additions to the histologic details of diseased kidneys. The reviewer, for one, is grateful to the author and to the Josiah Macy Jr Foundation, which has supported his studies and made possible this publication.

The book deals with the study of diseased kidneys by microdissection and reconstruction methods, supplemented and controlled to some extent by ordinary histologic studies.

A rapid perusal of the book gives one the impression of a vast amount of painstaking work, done honestly and presented on the whole dispassionately. As might be anticipated, the number of details encountered in these studies was great and the difficulties of presentation correspondingly difficult. The author is to be complimented on his manner of approach and his success in exposition.

Rereading, with careful consideration of the author's discussions and such correlations as he makes with what is known about the pathologic physiology of the kidney, convinces the reviewer that significant new facts are established and that the importance of the volume is great because of the stimulus it provides for the imagination. The illustrations throughout the book are superb and achieve their purpose. A number have been taken from previous studies of Oliver and his collaborators, published in the *ARCHIVES OF PATHOLOGY* in 1933, 1934 and 1935, but these are few in comparison with the additions.

The first chapter is devoted to "Technique and Methods. Material and Method of Presentation."

The second, third and fourth chapters present objectively the structural changes encountered in glomeruli and tubular systems in the various forms of Bright's disease.

Chapter 2 is concerned with the two outstanding features of chronic kidney disease, hypertrophy of the nephron (glomerulo-tubular unit) and atrophy of the nephron. Results are illustrated from reconstructions.

Chapter 3, "The Morphology of the Abnormal Nephron," describes the glomerulus and various parts of the tubules as encountered in terminal Bright's disease. Most of the examples were taken from glomerular nephritis, a few, from arteriosclerotic nephrosclerosis. The purpose of the chapter is to present the complete range of abnormalities found, with emphasis on the fact that the typical morphologic changes are the same in all forms of the renal lesion. The many illustrations are from photographs of microdissections.

Chapter 4 is entitled "The Agglomerular Nephrons of Terminal Bright's Disease" and establishes the fact that tubules may persist after destruction of their glomeruli. Good evidence is presented that such tubules are functional. The illustrations are from microdissections and reconstructions and fully document the author's conclusions.

Chapter 5 is a short one, entitled "The Transformation of the Arterial System in Terminal Bright's Disease." It is illustrated chiefly from microdissections. It demonstrates the formation of new vascular channels which serve to make the tubules independent of the passage of blood through the glomerulus. The vessels

are (1) Ludwig's vessel, which arises from the afferent arteriole and breaks up into the intertubular network, (2) a branch which develops from the interlobular artery and ends in the intertubular capillaries and (3) branches which arise from the arcuate artery or the deep portion of the interlobular artery and descend in the medulla. Such descending arteries may also take origin from Ludwig's vessel.

The sixth chapter, "Factors Responsible for the Regressive Alterations of the Kidney Parenchyma in Terminal Bright's Disease," discusses destruction of glomeruli as a cause of tubular atrophy and arterial disturbances responsible for nephron regression. These two parts of chapter 6 are informative and convincing. The third part of chapter 6—"The Interstitial (Inflammatory) Reaction As It Affects the Parenchymal Elements"—is the weakest part of the book and perhaps is the only part which can be considered to provide bait for disputative-minded pathologic histologists. It is the only part in which the author has indulged to a slight degree in interpretative comments—something which he waimly deprecates in his consideration of much of the current pathology of the kidney. On the whole, the reviewer thinks it is somewhat regrettable that the word "inflammatory" has been used in regard to the behavior of the interstitial tissue of the kidney in view of the different connotations aroused by that term in the minds of thoughtful pathologists. However, part 4 of this chapter, devoted to "Obstructive or the Hydronephrotic Factor in Nephron Distortion," again brings objective details valuable as premises for thoughtful speculation.

Chapter 7, in which the author takes up "The Pattern of Kidney Structure in Bright's Disease," is well presented and illustrated by a selection of objective findings of great beauty, obtained from cases studied and presented in the book by Addis and Oliver, published in 1931, entitled "The Renal Lesion in Bright's Disease" (Paul B. Hoeber, Inc., New York). The general plan is to present microdissections on one page and a histologic section on the opposite page, with a brief resume of the clinical history of the patient and the histologic observations. The author has taken great pains and has shown ingenuity in the use of devices in his illustrations in order to give information concerning details of pathologic alteration of the nephron and the blood vessels.

There is a final chapter—the eighth—entitled "Epicrisis and Prolegomenon"—which consists of only 9 pages in which the author expresses rather more optimism in regard to the possibility of correlating the details he describes with function. Rightly, he insists that further advances in knowledge require collaboration by pathologists and clinicians competent in physiologic methods of study. Perhaps one might say that occasionally he goes a bit too far in assuming that alterations in structure are indicative of corresponding qualitative changes in function.

A third reading of the book has convinced the reviewer that wherever the author has exposed himself to adverse criticism there is occasion only for sympathetic and respectful toleration, because it is evident that in the performance of such a large amount of work many observations must have been made (known only to him) that are not now amenable to analysis and presentation.

To review this book adequately would require a great deal of space, and it is doubtful if it can be done satisfactorily without reproduction of some of the illustrations. The reviewer believes that the material is so beautifully and clearly presented that well informed students of nephritis can make use of it for their own purposes and may very well come to conclusions different from those expressed by the author.

The actual contributions that Oliver has made are extraordinarily impressive: (1) the demonstration that tubules persist and apparently are functional after the glomerulus is completely destroyed, (2) that tubules with intact glomeruli, as well as aglomerular tubules, undergo hypertrophy, (3) the demonstration that in the course of nephritis important circulatory changes take place in the kidney—for example, the development of Ludwig's arteriole and the establishment of other arterial supplies to the tubular network from the interlobular arteries, (4) the



varieties of morphologic change that may occur in the tubule of a single nephron, (5) the obstructive role of material within the tubule, and (6) the change in the pattern of the blood supply in vascular nephritis

All pathologists interested in diseases of the kidney or forced, in their routine, to write descriptions of diseased kidneys should have this book. The reviewer has already derived great satisfaction in his better understanding of microscopic slides of diseased kidneys. The physiologically trained clinical investigator of nephritis will also find this book of value. It will make clear to him the difficulties of the pathologist and should provide some new ideas for methods of study during life.

Dr. Oliver makes no claim for contributions on the genesis of Bright's disease, nor is it apparent that the methods he has used are adapted for this objective. The importance for physiology through encouragement of research on the physiology of the tubule is apparent. While there is some doubt in the mind of the reviewer of the probability of bringing to light by these methods other facts approaching in importance those already presented by Oliver, he has no hesitancy whatever in proclaiming this book to be outstanding in importance, one that will materially influence the trend in investigations of the physiology and pathology of the kidney.

**Electrocardiographic Patterns** Arlie R. Barnes, M.D., The Mayo Clinic, Rochester, Minn. Cloth. Pp. 197, with 94 illustrations. Price \$5. Springfield, Ill., and Baltimore, Md.: Charles C. Thomas, Publisher, 1939.

This excellent book represents an extraordinary amount of clinical, experimental and pathologic work in electrocardiography since 1928. In the fifteen years which have elapsed since the publication in 1925 of Sir Thomas Lewis', "The Mechanism and Graphic Registration of the Heart Beat," one can only be amazed at the tremendous amount of new material which Barnes (and other workers in this field, largely Americans) have contributed. Barnes, working with Whitten in 1928, "became interested in the possibility of working out correlations of electrocardiographic changes with specific pathologic conditions in the heart." Within certain limitations and with occasional exceptions the author believes, and this reviewer believes, they have succeeded, thus fulfilling Herrick's prophetic question of 1919: "May it perhaps be possible to localize a lesion in the coronary system with an accuracy comparable to that with which we locate obstructive lesions in the cerebral arteries?"

The book opens with a clear description and with appropriate drawings of "the relation of the distribution of the coronary arteries to acute myocardial infarction," showing that, contrary to widespread belief, infarction occurs with about equal frequency in the anterior-apical and posterior-basal portions of the left ventricle, although occlusion of the anterior descending branch of the left coronary artery occurs more commonly than does occlusion of the right coronary artery. Barnes emphasizes that the resulting electrocardiographic pattern reflects the *site* of infarction of the left ventricle (not the *vessel* occluded), and later he offers certain explanations as to the extraordinary infrequency of infarction of the *right* ventricle.

Chapter 3 discusses the "electrocardiogram in acute myocardial infarction and in its healing stages," differentiating the so-called  $Q_1T_1$  patterns from the  $Q_1T_2$  patterns, etc. One important comment is made regarding the value of serial electrocardiograms in the diagnosis of acute and healing myocardial infarction, namely, that other factors, such as "acute pericarditis, acute cor pulmonale, diabetic acidosis, digitalis therapy, certain acute infections, may likewise produce a tracing which differs from day to day."

In contrast to the usefulness of the electrocardiographic patterns in the diagnosis of myocardial infarction, attention is called to "the role of the electrocardiogram in the diagnosis of *nonocclusive* coronary disease." Noting that while many patients with advanced coronary sclerosis, even when it results in myocardial

fibrosis, have normal electrocardiograms, "organic delay in auriculoventricular conduction, and complete and incomplete bundle branch block are associated in a high percentage of cases with nonocclusive coronary sclerosis"

The electrocardiographic pattern of predominant strain of either ventricle, manifested at autopsy by ventricular hypertrophy and dilatation and during life by failure of the left or of the right side of the heart, is discussed in chapter 4. The electrocardiogram in *acute* right ventricular strain (acute cor pulmonale) is described in chapter 5, with particular reference to the role of pulmonary embolism and infarction as a classic example of unilateral ventricular strain. Chapter VI discusses the electrocardiogram in *chronic* right ventricular strain.

The electrocardiogram in pericarditis is considered in chapter VII, with many new contributions to present knowledge. One particularly interesting statement may be noted: "It is therefore obvious that the weight of evidence derived from experimental and clinical observation indicates strikingly that the electrocardiographic changes in pericarditis, in most instances at least, are an expression of the associated subepicardial myocarditis."

The book closes with chapters on the "effects of certain drugs, metabolic disorders and infections on the electrocardiogram," and "some observations relative to precordial leads." One feels that with the extensive references to pertinent literature, the complete author and subject index, the fine format of the book itself and the wide experience and sound clinical judgment of the author, this book constitutes a noteworthy contribution to an important and ever widening clinical and physiologic discipline.

## Books Received

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THIRTY-SEVENTH ANNUAL REPORT, 1938-1940, OF THE IMPERIAL CANCER RESEARCH FUND Founded under the direction of the Royal College of Physicians of London and the Royal College of Surgeons of England, 1902 Incorporated by Royal Charter, 1939 Paper Pp 43 London Taylor and Francis, 1940

THE FIFTY-FIFTH ANNUAL MEDICAL REPORT OF THE TRUDEAU SANATORIUM AND THE THIRTY-FIFTH MEDICAL SUPPLEMENT FOR THE YEAR ENDING SEPTEMBER 30, 1939, TOGETHER WITH THE TWENTY-THIRD COLLECTION OF THE STUDIES OF THE EDWARD L TRUDEAU FOUNDATION FOR RESEARCH AND TEACHING IN TUBERCULOSIS, 1939

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